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A MANUAL
OF
BACTERIOLOGY

CLINICAL AND APPLIED

WITH an APPENDIX on BACTERIAL REMEDIES &c.

BY

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P R E F A C E

IN the following manual I have endeavoured to give some account of those portions of Bacteriology which are of especial interest in clinical medicine and hygiene. The preparation of tissues, methods of culture, descriptions of pathogenic organisms and their detection, the examination of water, &c., have therefore been given at some length. As it would be impossible in the space at my disposal to include everything relative to the subject, a selection has had to be made, and such details as the celloidin method, Löffler's stain for flagella, the strictly animal parasitic diseases (with a few exceptions), &c., have, among others, been omitted.

At the end of the sections dealing with the pathogenic organisms which attack man, some directions have been given for the bacteriological clinical diagnosis and examination, but these are in no way exhaustive; in fact, it would not be possible in a short work to give a scheme of examination which would cover every case. These directions will also render the book of service in the laboratory, while I venture to hope that the details given in the Appendix on the

use of the remedies and diagnostic agents of bacterial origin may be of value to the practitioner.

I have to thank Mr. PEYTON BEALE, Dr. LAMBERT LACK, and Mr. F. J. TANNER for suggestions and criticism, and the last-named gentleman for the aid he has freely given me in the revision of the proof-sheets. I am also indebted, indirectly, in many ways to my colleagues, Dr. MACFADYEN and Mr. FOULERTON. My thanks are due to Mr. J. BARNARD and to Mr. FRANK STRATTON respectively for the photo-micrographs and original drawings, while for the eight borrowed illustrations blocks have been kindly lent by Messrs. BAIRD and TATLOCK, and Messrs. SWIFT & SON.

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A MANUAL OF BACTERIOLOGY

INTRODUCTION.

BACTERIOLOGY is that branch of Biology which deals with the study of Micro-organisms, both animal and vegetable. The scope of bacteriology is difficult to exactly define, the term being now used in a comprehensive sense equivalent to micro-pathology, or even micro-biology, for all investigations connected with micro-organisms are included under it. Bacteriology, however, especially deals with micro-organisms in their relation to processes—disease, fermentation, putrefaction, and the like—while their structure, functions, and life-history are to a large extent left to the botanist and zoologist. There is no space in a work of this kind to enter into the history of the science, but the names of Leeuwenhoek (1675), Müller (1786), Schwann (1837), Cohn, Pasteur, Lister, and Koch will ever hold an honourable place in its annals.

The study of micro-organisms must always be of considerable importance in general biology; their vital phenomena are comparatively simple, and throw much light on the complex processes occurring in the higher orders of living beings. Weismann bases his theory of heredity on the fundamental truth of the immortality of these unicellular organisms. Setting aside accidents, they are immortal—they

reproduce themselves by a process of simple division, an individual dividing into two, and two daughter forms take the place of the original parent one, and although the parent has disappeared yet there has been no death, no dissolution; its bioplasm or living material is still existent in its progeny and is immortal, since this process of reproduction may go on apparently indefinitely. Moreover, the study of the possibility of the transformation of species of micro-organisms is likely to throw light on the theory of evolution. Organisms such as bacteria, multiply so rapidly, that fifty or sixty generations may be developed in thirty hours, a number which would take a lifetime to attain if even the most rapid breeder among mammals were the subject of experiment, and as they occur in vast numbers there is a wide field for variation. These are some of the points of the relation of micro-organisms to general biology.

In what may be termed the economy of nature micro-organisms are all-important, without them there would be no putrefaction, no decay, and the dead remains of animal and vegetable life would so accumulate as to encumber the earth, which would become sterile for the want of the organic matter derived from it, but of which there was no return. In fact the higher plants, and indirectly, therefore, animals also, are dependent for their existence on the presence of bacteria in the soil, which break up and render assimilable complex substances presented to them as manure.

Commercially micro-organisms are of the utmost importance. Without them there would be no fermentation, and the wine, beer, and indigo industries, the ripening of cheese and tobacco, and many like processes would be non-existent. From a financial aspect also micro-organisms cannot be ignored, for many of the so-called diseases of beer and wine, which often occasion great loss, are due to the entrance of adventitious forms, while the silk industry and sheep farming in France were once threatened with extinction owing to

the ravages of pébrine and anthrax respectively, but were successfully combated by Pasteur. There is no need to emphasise the importance of micro-organisms from a medical and hygienic point of view, but the fact may be recalled that fifty or sixty years ago the mortality after operations was very high, and that 40 per cent. of these deaths were caused by pyæmia, septicæmia, and hospital gangrene, conditions which are due to the entrance of micro-organisms, and which are now almost preventable, thanks to the antiseptic system introduced by Lister.

The theory of spontaneous generation or abiogenesis is intimately connected with the study of bacteria. The putrefaction of animal and vegetable fluids even after boiling, and the growth in them of minute living forms, were held by many to be a sure proof of the development of life from inanimate matter, of the spontaneous generation of the living from the non-living. A succession of investigators, however, showed (1) that if the fluids were boiled sufficiently long, and then sealed up so as to prevent the access of air, they did not undergo putrefaction; (2) that the sealing up could be dispensed with, provided the air were first filtered through cotton-wool before being admitted to the flasks; (3) that even the cotton-wool was not needed if the air were passed slowly through a long and tortuous channel, so as to deposit its solid particles. Tyndall showed that putrescible fluids might be left in open vessels in a closed chamber in which the air had been undisturbed for some time and its solid particles thereby deposited on the walls of the chamber, which had been smeared with glycerine; he also proved that vegetable infusions and the like, which putrefied after having been boiled for ten minutes, did not do so if the boiling were repeated on two or three successive days, and explained this by the supposition, that while the fully developed bacteria were destroyed by the first boiling, their more resistant spores on being left for twenty-four hours germinated into the

less resistant bacterial forms, which were destroyed by the second boiling, and by the repetition of the process complete sterilisation was ultimately obtained. It is this process of 'discontinuous sterilisation,' as it is termed, which is employed by the bacteriologist for the preparation of sterile culture media.¹

Doubtless immense progress has been made during the last two or three decades, but a vast amount still remains to be done. We have only touched the fringe of the explanation of the difficult problems of immunity, of the extraordinary variations in virulence and effects of the same organism, and of the important question of cure in, and prevention of, infective diseases, while the chemistry of the products of bacterial activity is but in its infancy. There is still room for a host of workers, not only for fresh conquests but to review the work already accomplished, for in this age of haste and the rush to be first many imperfect results and too hasty generalisations have been published.

The literature of Bacteriology is now becoming somewhat extensive. In the following pages a good many references to original papers have been introduced, so that further information may be obtained if desired, the aim being as far as possible to refer to easily accessible papers containing a more or less full bibliography on a particular subject.

The English journals containing papers on bacteriology are the 'Journal of Pathology and Bacteriology,' 'The Lancet,' 'The British Medical Journal' and its useful epitome, and the provincial medical journals; 'The Medico-Chirurgical and Pathological Societies' Transactions, the 'Proceedings and Transactions of the Royal Society' of London, the 'Journal of the Royal Microscopical Society' (abstracts), the 'Journal of State Medicine' (hygienic), the 'Journal of Comparative Pathology and

¹ The writer believes that this explanation is only partially true, and would ascribe some of the sterilising effect of repeated heatings simply to the injurious action of alternate heating and cooling.

Therapeutics' (veterinary), and the 'Brewers' Journal' (fermentation &c.). The chief American medical Journals and the 'Journal of Experimental Medicine' (Baltimore) also contain valuable bacteriological papers. The French journals are the 'Annales de l'Institut Pasteur,' and the 'Annales de Micrographie' (abstracts), and various medical journals. In German, the 'Centralblatt für Bakteriologie und Parasitenkunde' (both parts), the 'Zeitschrift für Hygiene,' 'Hygienische Rundschau,' and the medical journals generally. Baumgarten's annual 'Jahresberichte' (pathogenic organisms) and Koch's annual 'Jahresbericht' (fermentation &c.) give valuable epitomes, but the papers referred to are a year or two old.

A very full bibliography is given in Sternberg's 'Manual of Bacteriology' (1893), and in the recently published 'Die Microorganismen' edited by Flügge. The volumes of the 'Index Catalogue' of the Surgeon-General's Library at Washington may also be consulted.

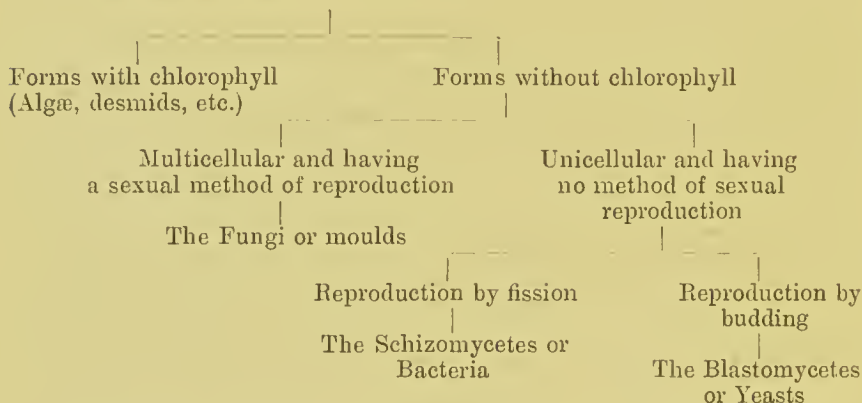
An index to current literature will be found in the 'Index Medicus,' published monthly.

CHAPTER I.

THE NATURE, STRUCTURE, AND FUNCTIONS OF THE BACTERIA :
THEIR CLASSIFICATION, GENERAL BIOLOGY, AND CHEMISTRY
—BACTERIA AND DISEASE.

THE Bacteria or Schizomycetes ('fission fungi') are minute vegetable organisms for the most part unicellular and devoid of chlorophyll. A certain number of filamentous forms are also included, serving to connect the unicellular ones with the multicellular fungi. Their relation to the other lower plants can be seen in the following scheme :—

Thallophyta (lower plants without fibro-vascular bundles, and with no distinction between root and stem)



Their size is variable, but they are all microscopic, measuring from 0.3μ to $30-40\mu$ in diameter or length. Their shape likewise is very different in the different species ; some are spherical, others ovoid, others rod-shaped or filamentous, while in some the rod or filament is twisted into a spiral.

We still know little of the intimate structure of the bacterial cell; it consists of a cell-membrane enclosing the transparent, more or less structureless living matter or bioplasm.¹ The cell-membrane in some species seems to be composed of cellulose, but in the majority it appears to be but a condensed layer of the bioplasm. The bioplasm is generally granular in appearance, and frequently contains larger granules composed of fatty or proteid matter, pigment, and in some species of sulphur.

The presence of a nucleus is uncertain, but by special methods of treatment the bioplasm can be differentiated into an outer layer surrounding an inner portion or kernel, which perhaps corresponds to a nucleus. The bioplasm when dead, according to Nencki, differs from proteid matter in not being precipitated by alcohol and in not containing sulphur; it has been termed by him 'myko-protein.'

The cell-membrane frequently becomes thickened, swollen, and gelatinous, forming a layer or so-called 'capsule' around the organism. When large groups of bacteria become thus encapsuled, they adhere together in a sort of jelly-like matrix, forming what is known as a 'zoogloea.'

All species of bacteria, especially the smaller ones, when suspended in a fluid exhibit what is known as Brownian movement, consisting of an oscillation with some amount of rotation about a fixed point, but there is no actual movement of translation. This Brownian movement is physical and not vital in nature, and occurs with all fine particles suspended in a fluid, and must be clearly distinguished from a true vital motility. Some bacteria are always motionless, others are more or less motile, but these, too, have a resting stage. For motility to occur the cells must be young, and the conditions favourable to growth and development.

¹ The writer has preferred to employ the term bio-plasm to designate that which is usually known as proto-plasm, though he is aware that it has been used by Lionel Beale in a more restricted sense.

Motility seems to be due to delicate bioplastic threads termed flagella connected with the outer layer of the cell bioplasm; these move actively and propel the organism through the medium. It is curious, however, that the cell will move indifferently in either direction; if a motile organism be watched it will often be seen to proceed rapidly in one direction, stop, and then return without turning round. The flagella are not visible in the living state, nor by the ordinary methods of staining, unless previously treated with a mordant. They vary considerably in number and length; some organisms have only one or two at either pole; others have several, and may be almost entirely covered with them; in some the flagella are short and straight, and in others long and twisted. The motility of organisms does not depend directly upon the number of flagella they possess, an organism with a few flagella being often more active than another possessing many, and some are apparently non-motile, though well-marked flagella can be demonstrated.

Darwin says, 'In looking at nature it is most necessary never to forget that every single organic being may be said to be striving to the utmost to increase in numbers,' and in no group perhaps of the animal and vegetable kingdoms is this more marked than among the bacteria. Reproduction is entirely non-sexual, which distinguishes the bacteria from the true fungi, and it takes place in two ways—by simple division or fission and by spore formation. Reproduction by transverse fission is common to all bacteria; the bacterial cell becomes constricted at its middle and finally separates into two parts, and thus two young cells take the place of the parent one; reproduction by fission is therefore also an increase in numbers. Under favourable conditions reproduction may be very rapid, fission occurring every 20 or 30 minutes (Klein), so that, the increase being in a geometrical ratio, the number of individuals which might arise from a single bacterium in three or four days is almost inconceivable,

and would *en masse* weigh thousands of tons ; fortunately, there are many checks to such a rapid multiplication. Frequently, although the bioplasm divides, the division of the cell-membrane is incomplete, resulting in a loose union of the cells with the formation of a pseudo-filament. These filaments often become much curved and twisted, forming tangled masses owing to fission taking place in the cells in the middle of the filament as well as at the ends, so that the filaments have to become curved to make room for the new cells. Shattock has suggested that in some rod forms fission may occasionally occur longitudinally as well as transversely, which would account for the marked parallel arrangement of bacterial cells sometimes met with.

Reproduction by spore formation is only met with in some species, and is of two kinds. In the first, 'endogenous' spore formation, a bright round or ovoid body is formed within the bacterial cell, the development of which can be watched under the microscope. The first change noticed is that the bioplasm becomes markedly granular ; then a bright point appears in it, which increases in size until the fully-grown, highly refractile spore is produced. The bioplasm of the cell afterwards degenerates, the cell-membrane ruptures, and the spore is set free. Spores are single, one only forming in each cell, and they seem to fulfil the purpose of perpetuating the race when it is threatened with extinction from adverse circumstances. The spore consists of a little mass of bioplasm enclosed within a very tough and resisting membrane, which preserves its vitality even under unfavourable conditions ; for spores resist the action of desiccation, heat, and germicidal agents to a much greater degree than the fully-developed organisms. Spores vary much in size and in the position they occupy within the bacterial cell in the different species ; their diameter is usually about the same as that of the cell in which they are developed, but it may be much greater, and in position they may be central or terminal, and

sometimes the spore-bearing cells are swollen or club-shaped. The second variety of spore formation, 'arthrospore' formation, is only of occasional occurrence, and the whole cell seems to partake in it. Some of the elements formed by fission are slightly larger, more refractile, and more resisting than their fellows, and appear to fulfil the function of spores. Placed under favourable circumstances, the spore in either case germinates, it becomes swollen and granular, and loses its refractile appearance; a slight protuberance forms on one side, this increases in size, and an organism similar to the parent one is finally reproduced, the empty spore membrane at first frequently enclosing one extremity, and then being cast off.

CONDITIONS OF LIFE OF BACTERIA.

Bacteria, being living organisms, must be supplied with suitable nutritive substances in order that their life-processes, nutrition, reproduction, and the like, may be carried on in a normal manner. Being devoid of chlorophyll they are mainly dependent on the higher organic compounds for the carbon, hydrogen, and nitrogen which enter into their composition, these elements being derived for the most part from proteids and carbohydrates. Some bacteria, however, are able to obtain the requisite nitrogen from such comparatively simple compounds as ammonia, ammonium carbonate, or nitrates, and one group can make direct use of the atmospheric nitrogen. Certain inorganic salts, sulphates, phosphates, and sodium chloride, also seem to be necessary for normal development. These nutrient substances must be presented to the bacteria in association with water, for without water bacterial activity ceases, though in the dry state many forms, and especially their spores, may retain their vitality for a considerable time; absolute desiccation, however, is probably rapidly fatal.

Temperature is also an important factor. Though the growth of many species occurs through a wide range, there is

for almost all an optimum at which growth is best, and of a range not exceeding 4 or 5 degrees. Growth usually ceases below 10°C. , but cold does not destroy bacterial life; after exposure to the intense cold produced by the evaporation of liquid oxygen (-180°C.) bacteria and their spores will grow and germinate (Dewar). On the other hand, bacterial growth usually ceases when the temperature exceeds 40°C. or thereabouts, and most bacteria without spores are destroyed in half an hour or so by a temperature of 65°C. The spores are far more resistant, some can even be boiled for a short time without losing their vitality, but prolonged boiling is fatal to both bacteria and their spores. There is, however, a group of so-called thermophilic bacteria, which thrive best at a temperature of 60° to 70°C. They occur in the soil and in water, and are probably of considerable importance in the natural fermentations accompanied by the evolution of heat, such as are met with in manure heaps, the heating of hay, and fring of moist cotton.¹

Free oxygen is essential to the growth of some organisms; these are termed strictly aërobic. Others will not develop in its presence, strictly anaërobic; others again, while preferably aërobic or anaërobic, will grow in the absence, or in the presence, of oxygen, and are respectively termed facultative anaërobic or facultative aërobic. Some organisms are strictly parasitic on animals or plants; others live in water, soil, decaying matter, &c., these are termed saprophytes; and many are able to exist as parasites or saprophytes—facultative parasites.

Bacterial development is much influenced by the presence of foreign substances in the nutrient medium. A number of bodies, metallic salts; chlorine, bromine, and iodine, carbolic acid, salicylic acid, &c. have an injurious effect upon bacterial life, inhibiting or stopping growth, or killing the organisms outright; these are of considerable practical importance and

¹ Macfadyen and Blaxall, *Journ. of Path. and Bact.* Nov. 1894.

are known as germicides, antiseptics, and disinfectants. The products produced in the nutrient medium by the bacteria themselves also sooner or later inhibit or stop their further growth; a familiar instance of this is seen in the alcoholic fermentation of sugar by yeast, which ceases when the amount of alcohol reaches 12 or 14 per cent. This is also probably the explanation why the growth of bacteria on our nutrient media does not spread all over the surface, and why our cultures sometimes die out more rapidly than would be expected.

Another point affecting bacterial life is the presence of a mixture of organisms in the same nutrient medium. If there be a very vigorous form, it may ultimately grow and multiply to such an extent as to crowd out and finally kill the other forms with which it is associated, and if the nutrient medium equally favour two species, that one which is in an excess at the beginning may crowd out the others. The occurrence of what has been termed symbiosis is of considerable interest in the life of micro-organisms, and to which too little attention has hitherto been paid. This may be defined as the co-existence of two or more species together for their mutual benefit—nutritive, defensive, &c. For example, in the well-known ginger-beer plant, Marshall Ward¹ has isolated several yeasts, bacteria and moulds, and finds that one of the yeasts and one of the bacteria together induce the particular changes in a saccharine fluid to which ginger has been added, which render the mixture like ginger-beer, these changes not occurring unless both species develop together.

Another extraordinary feature exhibited by bacteria is the selective action exerted on certain substances which contain isomerides or right and left-handed modifications of a substance. The *bacillus ethaceticus* attacks mannite but not dulcite, two sugars which are very similar in taste and properties and having the same chemical formulæ.

¹ *Phil. Trans. Roy. Soc. Lond.* 1892, vol. 183, p. 125.

By a series of most brilliant researches Emil Fischer has succeeded in determining the constitution of the various sugars, and, what is more, has produced them artificially in the laboratory. The natural sugars are all of them bodies with dissymmetric molecules, powerfully affecting the beam of polarised light, but when prepared artificially they are without action on polarised light, because the artificial product consists of equal numbers of left-handed and right-handed molecules, the molecules of the one neutralising the molecules of the other, and thus giving rise to a mixture which does not affect the polarised beam.

By the action of micro-organisms, however, on such an inactive mixture the one set of molecules is searched out by the microbes and decomposed, leaving the other set of molecules untouched, and the latter now exhibit their specific action on polarised light, an active sugar being thus obtained.

One of the principal artificial sugars prepared by Fischer is called 'fructose'; it is inactive, but consists of an equal number of molecules of oppositely active sugars termed 'lævulose.' One set of these lævulose molecules turns the plane of polarisation to the right, another set to the left, right and left-handed lævulose. The left-handed lævulose occurs in nature, while the right-handed lævulose, so far as we know, does not.

Now, on putting brewer's yeast into a solution of fructose, the inactive artificial product, the yeast organisms attack the left-handed lævulose molecules and convert them into alcohol and CO_2 , while the right-handed lævulose is left untouched.

The question of life, animal and vegetable, without bacterial activity is an important and interesting one. It would seem from the experiments of Duclaux¹ that the higher plants under ordinary circumstances are unable to

¹ *Comp. Rend.* T. 100, p. 66.

obtain nutriment unless the complex compounds, proteids, urea, and ammonium salts, which form the important constituents of many manures, are broken down into simpler ones through the agency of bacteria. He sowed seeds in sterile soil free from nitrates, nitrites, and ammonia, which was plentifully watered with sterile milk and solutions of sugar and starch. No changes occurred in these substances, and the seeds lost weight and the seedlings dwindled and died. It is otherwise with the higher animals, although Pasteur had expressed the opinion that their life would probably be impossible without the presence of bacteria in the intestinal tract. Nencki expressed the opinion that this idea of Pasteur's was an erroneous one, and his experiments in conjunction with Macfadyen and Sieber¹ showed that any considerable decomposition of the food by bacteria first takes place in the large intestine, and that the digestive juices alone, without the co-operation of bacteria, are able to prepare the constituents of the food for absorption. Recent experiments by Nuttall and Thierfelder² settle the matter. Having obtained unborn guinea-pigs by Cæsarian section with antiseptic precautions, they afterwards kept them in a sterile environment and fed them on sterilised food. Not only did the animals live, but they were even in a more thriving condition than those naturally brought up. The intestinal tract was found to be sterile on the eighth day.

Pressure has little effect on bacteria, unless very great. Roger has investigated the effects of high pressure on certain organisms in bouillon cultures. Pressures of 200 to 250 kilos. per square centimeter had no effect; by raising the pressure to 3,000 kilos. per square centimeter one-third of streptococci were killed, and of anthrax without spores a good many; while sporing anthrax, *S. pyogenes aureus*, and the colon bacillus were unaffected.

¹ *Journ. of Anat. and Physiol.* xxv. p. 390.

² *Zeitschr. f. Physiol. Chem.* xxi. p. 109.

Our countrymen Downes and Blunt first called attention to the injurious effect of light on bacteria. If plate cultures be prepared and exposed to sunlight, a portion of the plate being protected from its action, as by sticking on a letter cut out of black paper, and the preparation afterwards incubated, it will be found that the colonies develop at the protected portion only, those parts which have been exposed to sunlight remaining sterile. Although this action of sunlight may occasionally be due to chemical changes in the medium, resulting in the production of ozone or other germicidal bodies, the experiments of Marshall Ward and others have conclusively shown that germicidal action may be caused by the direct action of the light, the violet and ultra violet rays being those concerned, and the red end of the spectrum having little effect. The Röntgen rays have no influence on bacteria.

Electricity, *per se*, has also usually little effect. When the current is passed directly through the cultures electrolysis takes place, and the products produced may destroy the bacteria; currents of high potential, however, may inhibit growth.¹

Living motile bacilli are very sensible to induced currents of electricity, immediately orientating themselves in the direction of the current, while dead or paralysed bacilli are unaffected.²

The length of time pathogenic bacteria retain their vitality in buried corpses has been the subject of experiment by Lösenner,³ who injected cultures into the bodies of pigs, which were then wrapped in linen, placed in wooden coffins, and buried. The conclusions he has arrived at are that, provided the soil has good filtering properties, there is practically no chance of the dissemination of a virus.

¹ LORTET, *Comp. Rend.* T. 119, 1894, p. 463.

² *Comp. Rend.* T. 122, 1896, p. 892.

³ *Centralbl. f. Bakt.* (1^{te}. Abt.) xx. 1896, p. 454.

CLASSIFICATION OF THE BACTERIA.

Many classifications of the bacteria have been proposed, but none up to the present can be said to be strictly scientific, or even satisfactory from the point of view of convenience. In the first place, the bacteria are said to be devoid of chlorophyll, but there are many forms intermediate between those unicellular organisms with and those without chlorophyll, so that a hard and fast line cannot be drawn. In the next place, the bacterial cells are so minute, and their vital phenomena so simple, that only a few broad distinctions can be observed in their morphology and reproductive processes.

One of the most prominent of the older classifications was that of Cohn. He divided the bacteria into four main groups :

- I. The sphærobacteria or spherical forms.
- II. The microbacteria or short rod-forms.
- III. The desmobacteria or long rod-forms.
- IV. The spirobacteria or spiral forms.

Zopf's classification (1885) has many points to commend it, but is largely based on the doctrine of pleomorphism. By pleomorphism is meant a variety or succession of morphological phases during the life-history and under different conditions, an organism, for example, being at one time a coccus, at another a rod, or a straight rod becoming a spirillum. In a peach-coloured bacterium examined by Lankester, cocci, rod, filamentous and spiral forms occurred, and the doctrine of pleomorphism received considerable support from his work ; but some doubt has recently been thrown on it, and it is suggested that he was not working with pure cultures. Be that as it may, a certain amount of pleomorphism undoubtedly occurs in some organisms. In the colon, typhoid and plague bacilli for example, the rods may be sometimes so short as to be almost cocci, while at others they are well-marked rods and even filaments. The following is an outline of Zopf's classification, the bacteria being

divided into four main groups or families, which again are subdivided into smaller groups or genera:—

Group I. COCCACEÆ.—Spherical forms only; division occurs in one or more directions.

Genus 1. MICROCOCCUS (Staphylococcus).—Division in one direction only, but irregular, so that the cocci after division form irregular clusters.

Genus 2. STREPTOCOCCUS.—Division in one plane, but regular, so that the cocci form chains.

Genus 3. MERISMOPEDIA.—Division in two directions at right angles to each other, but in the same plane, so that lamellæ or plates are formed.

Genus 4. SARCINA.—Division in three directions at right angles to each other and in two planes, so that cubical masses are formed.

Genus 5. ASCOCOCCUS.—Cocci which develop in a gelatinous matrix.

Group II. BACTERIACEÆ.—Rods, straight or curved, at some period of the life-history, though cocci and other forms may occur.

Genus 1. BACTERIUM.—Straight rods, Endospore formation does not occur.

Genus 2. BACILLUS.—Straight rods, Endospore formation occurs.

Genus 3. LEUCONOSTOC.—Cocci and rods, Arthrospore formation occurs in the cocci forms.

Genus 4. CLOSTRIDIUM.—The same as bacillus, but the spore-bearing rods are enlarged, and club-shaped.

Genus 5. SPIRILLUM.—Spiral rods, spore formation does not occur.

Genus 6. VIBRIO.—Spiral rods, spore formation occurs.

Group III. LEPTOTRICHEÆ.—These are all thread forms.

Group IV. CLADOTRICHEÆ.—These are thread forms provided with false branching.

There are many points in this classification which are of practical value. The distinction made between a bacterium and a bacillus, for example, is very convenient. Formerly it was the custom to term a short rod a bacterium, and a long rod a bacillus, but such a division is an arbitrary one, and at one stage of its life-history an organism might have to be termed a bacterium, and at another a bacillus. Recently the term bacterium has been little used, and any straight rod is termed a bacillus. The term staphylococcus is one frequently met with; it is practically synonymous with micrococcus, and refers to cocci which are aggregated into groups or clusters.

The latest system of classification is that proposed by Migula.¹ The bacteria are divided into five families (1) Coccaeæ, (2) Bacteriaceæ, (3) Spirillaceæ, (4) Clamydobacteriaceæ, and (5) Beggiatoaceæ. These, again, are subdivided into many genera, based partly on the mode of division and partly on the number and arrangement of the flagella of the organisms. For example, the Bacteriaceæ are defined as long or short cylindrical rods, straight and never spiral; division in one direction only after elongation of the rods; and this family has three genera (*a*) Bacterium, non-motile cells, often with endospore formation; (*b*) Bacillus, cells possessing both lateral and polar flagella, often with endospore formation; (*c*) Pseudomonas, cells with polar flagella only, rarely endospore formation.

In this manual the following terms will be employed—micrococcus (and diplococcus), streptococcus, and sarcina, clostridium, cladothrix, and leptothrix, in the same sense as in Zopf's classification; bacillus will include all rod, and spirillum all spiral forms, while streptothrix is applied in a general sense to all thread forms.

¹ *System der Bakterien*, 1897. This gives a full account and bibliography of the morphology and classification of the bacteria. Abstract in *Centr. f. Bak.* (1^{re} Abt.) xxii. 1897 (September), p. 345.

BACTERIAL PRODUCTS.

The chemical changes produced by micro-organisms are chiefly analytic or destructive, the formation of simpler from more complex bodies. This analytic faculty is present to a marked degree in what is known as putrefaction. *Putrefaction* is a term applied to the decomposition of organic, especially proteid, matter after the death of the animal or plant. It is usually accompanied by the evolution of foul-smelling gases and by the solution of the solid material. A large number of organisms are concerned in this, especially a group to which Hauser gave the name of proteus. The first changes which occur are the formation of proteoses and peptone. Then leucin, tyrosin, and glyocol, and basic compounds to which the name of ptomine has been given, next indol, skatol, and phenol, and volatile fatty acids, and lastly mercaptans, sulphuretted hydrogen, marsh gas, ammonia, carbonic acid, and hydrogen.

In view of its practical importance in bacteriological analysis and the identification of species, indol may here be referred to at some length.

Indol.—Indol (C_8H_7N) is formed by the growth of many organisms and is of considerable practical importance to the bacteriologist, as its detection or otherwise forms a valuable distinction between some organisms which resemble each other. Its recognition is based on the reaction with nitrous acid, which gives a fine purplish-red coloration. In order to test for it, the organism is grown in a fluid medium for twenty-four to forty-eight hours, a small quantity of nitrite of soda or potash is added, and then cautiously five or six drops of pure concentrated sulphuric acid, allowing it to trickle slowly down the side of the test tube, which is inclined with its mouth away from the operator. As the sulphuric acid runs

down, it is mixed with the fluid to prevent charring, which would result if a layer were allowed to form at the bottom of the tube. A colour varying from pale pink to pale purple indicates the presence of indol. A control tube, uninoculated, should also always be similarly tested to make sure that the reaction is due to the products of the growth of the organism. The culture fluid usually employed is peptone water, but some samples of 'peptone' occasionally fail to give the indol reaction when organisms are grown in media prepared from them; this is therefore another point to be ascertained—that the right kind of peptone is used. The amount of nitrite to be added is very small. A 4 per cent. solution of potassium nitrite should be kept in stock; 2 c.c. of this solution are diluted to 100 c.c. with distilled water at the time of making the test, and 1 c.c. of this dilution is added to every 10 c.c. of the culture. The addition of the sulphuric acid liberates free nitrous acid, which reacts with any indol present, and yields a pink colour. Sufficient sulphuric acid should be added to make the mixture moderately hot, and sometimes when apparently absent or feeble the reaction is obtained or intensified by placing the tube in the blood-heat incubator for half an hour.

Peptone water is by no means a good culture medium, and broth may therefore be employed, but it should be free from dextrose. Broth prepared in the ordinary way usually contains a little dextrose derived from the glycogen in the meat.

Gorini¹ and Kruse² state that the presence of dextrose, saccharose, or lactose in quantity exceeding about 0.25 per cent. prevents the formation of indol in broth by bacteria. This probably explains why the indol reaction is generally much more marked in a peptone water than in a broth

¹ *Centr. f. Bak.* xiii. 1893, p. 790.

² *Zeitschr. f. Hyg.* xvii. 1894, p. 1. See also T. Smith, *Journ. of Exper. Med.* ii. 1897, p. 543.

culture, although the latter is a better nutrient soil. In order to prepare a soil free from dextrose, T. Smith recommends that the acid beef broth used in the preparation of nutrient broth should be inoculated with the colon bacillus and incubated for twenty-four hours, then filtered through porous porcelain, and the peptone beef broth prepared from it. The dextrose is consumed and no indol is formed.

Some bacteria not only form indol but also produce nitrites in the culture medium by the reduction of the nitrates present in the peptone, &c., used in making the nutrient medium, in which case the addition of pure sulphuric acid alone is sufficient to bring out the pink indol reaction. This forms, therefore, an additional means of distinguishing organisms, and is employed especially for the recognition of the cholera spirillum. This organism if grown in peptone water gives the indol reaction (or, as it has been termed, 'the cholera red reaction') on the addition of sulphuric acid alone; it can be obtained as early as twelve hours after inoculation, and is very marked in twenty-four to forty-eight hours.

Unless the sulphuric acid be pure and free from oxides of nitrogen, hydrochloric acid should be employed.

The most delicate method of testing for indol is to run a little hydrochloric acid down the side of the tube, so that a layer forms at the bottom, the nitrite having been previously added to the culture if required. A pink ring at the juncture of the hydrochloric acid and culture indicates the presence of indol. The pink pigment, the product of the reaction, may be extracted by shaking with a little amylic alcohol.

Another important series of changes is that included under the term nitrification. As mentioned before, proteid, albuminoid, and other nitrogenous matters and ammonium salts, all of which are valuable manures for plant life, cease to be so unless bacteria are present.

Through the agency of the putrefactive bacteria ammonia

compounds are ultimately produced from the more complex nitrogenous substances. In order, however, that plants may obtain their requisite nitrogen, the nitrogen must be in the form of nitrates.

Although so important, extremely small quantities are present in the soil; in fertile soils, for example, there may be under some conditions as little as one part of nitrogen in 1,000,000, and there is often less than ten parts. The bodies yielding nitric acid in the soil are: (1) free nitrogen, (2) small quantities of nitrates in rain-water, (3) ammonium salts, applied intentionally or carried to the soil by rain or derived from the decay of organic matter, (4) various nitrogenous organic substances arising from the decay of animal and vegetable matters.

With regard to the production of nitric acid from nitrogenous organic matters very little was formerly known. In 1877 Schloesing and Müntz by an ingenious experiment showed that nitrification (as the production of nitric acid is termed) of nitrogenous organic matter is brought about by living organisms in the soil. A tube one metre long was filled with a mixture of 5 kilos. of ignited quartz sand and 100 grams of limestone. To this sewage was supplied at such a rate that it took eight days to pass through. During the first twenty days the sewage passed through unaltered; after that nitric acid began to appear in the filtrate, and increased to such an extent that the filtered sewage contained no ammonia but nitrates only. After passing sewage for four months with complete oxidation of ammonia, the authors placed a vessel of chloroform so that the vapour poured into the tube. In ten days after the introduction of the chloroform all nitrates disappeared in the exit water, and the sewage passed through unchanged.

After fifteen days the vessel containing the chloroform was removed. Nitrification did not resume, and after seven weeks the washings from ten grams of garden soil were

added. Eight days after this addition nitrates again appeared in the exit water (this was confirmed by Warington).

Shortly after this Schloesing and Müntz found that exposure of soil to 100° C. for an hour destroyed the power of nitrification. Soils thus treated were exposed to a current of air, purified by ignition, without nitrification taking place; the addition of a little unheated mould was sufficient to cause nitrification to recommence. They also tried seeding the sterile soils with various moulds &c. without result.

In 1884 Warington concluded that the factor determining the formation sometimes of nitric acid and sometimes of nitrous acid was a difference in the character of the organism; for it is possible to have two similar solutions under identical conditions, and in the one only nitrites, and in the other only nitrates are produced.

In 1886 Dr. Munro showed that the process of nitrification could take place in solutions practically destitute of organic matter.

The methods of isolation and the nature of the organisms of nitrification must next be considered. Nitrification in the soil takes place in three stages:

I. *Ammonisation*.—When complex organic compounds such as albuminoids are applied to the land they are broken up; first they become liquefied, peptone-like bodies being produced; these are then further acted upon and we get alkaloidal substances in small quantity, indol, skatol, leucin, and tyrosin, valerianic acid, volatile fatty acids, lactic acid, &c.

These changes are brought about by numbers of organisms, among which the varieties of proteus (formerly known as *bacterium termo*) are the more important.

Then the nitrogenous compounds are further acted on and ammonium salts formed. According to Emile Marchal, ammonisation takes place essentially under the influence of microbes living in the upper layers of the soil. The *bacillus*

mycoides is one of the most energetic of these, and seems to play a double rôle, being ammonising both in the presence of nitrogenous organic substances and of nitrates. Urea is ammonised especially by the *micrococcus ureæ*.

II. *Nitrosation*.—The ammoniacal salts are next converted into nitrites. The nitrous organisms probably can attack nitrogenous organic substances such as asparagine and milk, but only feebly, milk being much more rapidly nitrified when the nitrous organisms are mixed with other organisms.

III. *Nitratation*.—These nitrites are then converted into nitrates.

There is accumulated evidence to show that stages II. and III. are brought about by different species, the nitric organisms having no effect whatever on ammonia, but only acting after this has been oxidised into nitrous acid.

The discovery of Dr. Munro that organisms would grow in purely inorganic solutions has been made use of for the isolation of the different species. Solutions such as the following have been used :

For the Nitrous Organisms.

Ammonium chloride 0·5 gm.
Potassium phosphate 0·1 gm.
Magnesium sulphate 0·02 gm.
Calcium chloride 0·01 gm.
Calcium carbonate 5·0 gm.
Distilled water 1,000 c.c.

For the Nitric Organisms.

Potassium nitrite 0·3 gm.
Potassium phosphate 0·1 gm.
Magnesium sulphate 0·05 gm.
Calcium carbonate 5·0 gm.
Distilled water 1,000 c.c.

These are seeded with traces of earth, and by carrying on the cultivation for many generations a large number of organisms are eliminated. This method does not lead to a pure cultivation, for several forms besides the nitrifying organisms persistently maintain themselves in these mineral solutions.

So recourse was had to gelatin plate cultivations. Although several organisms were isolated in this manner, none of them possessed the slightest nitrifying power.

Frankland, and later Warington (1890), succeeded in isolating nitrous organisms by the dilution method. Nitrifying solutions were diluted, and traces inoculated into ammoniacal solutions; in some of these nitrification occurred, although no growth could be obtained on gelatin, and they were found to contain the nitrous organism only. A little later Winogradsky isolated nitrous organisms, first by modified gelatin plates, and afterwards by the silica jelly method.

Warington gives the following directions for the preparation of silica jelly plates: Sodium carbonate is fused in the blowpipe, fine white sand is then added as long as effervescence is produced. The mass is allowed to cool, and is then dissolved in water. The solution is poured into an excess of very dilute hydrochloric acid (silicic acid and sodium chloride being formed). The solution is dialysed and sterilised. Some of this is placed in a sterile dish and mixed with the following solution and inoculated:—

Ammonium sulphate	. . .	0.4 gram.
Magnesium sulphate	. . .	0.5 gram.
Di-potassium hydrogen phosphate	. . .	0.1 gram.
Calcium chloride	. . .	trace.
Sodium carbonate	. . .	0.6–0.9 gram.
Water	. . .	100 c.c.

This mixture sets to a jelly in five to fifteen minutes.

The organisms isolated by Warington, Frankland, and Winogradsky were all nitrous forms.

Warington and Winogradsky in subsequent attempts succeeded in isolating a nitric organism. It occurs in much the same form as the nitrous, viz. as round or ovoid corpuscles. These organisms, while having an energetic action in converting nitrites into nitrates, are without action on ammonia—in fact, this is inhibitory.

The nitrification of the soil is thus brought about by two groups of organisms. The first oxidises ammonia into

nitrous acid, and is isolated by successive cultivation in solutions of ammonium carbonate. The second organism oxidises nitrous acid into nitric acid, and may be separated by successive cultivations in a solution of potassium nitrite containing a little sodium bicarbonate. In the soil the nitric organism is equally active as the nitrous.

The Leguminosæ, however, are able to obtain their nitrogen directly from the nitrogen of the air, also through the agency of micro-organisms. If the roots of a pea, bean, or vetch be examined numerous little nodules will be found upon them; on examining these microscopically small irregular bodies are found to be present, which have been termed bacteroids. On inoculation into culture media the bacteroids give rise to a growth of organisms resembling bacteria; these 'fix' the atmospheric nitrogen. Leguminous plants grown from sterile seeds in a sterile soil dwindle and die, but if seeded with the organisms derived from another plant of the same species growth becomes vigorous; if with those derived from another species growth still takes place, but not nearly to the same extent.

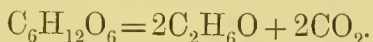
Another important group of changes produced by micro-organisms is that comprised under the comprehensive title of fermentation, of which it is difficult to give an accurate definition, for the distinction between it and other chemical changes due to the activity of micro-organisms is rather conventional than scientific. Fermentation is brought about by the action of ferments, two classes of which are recognised, viz. the living or organised ferments, which, in other words, are micro-organisms; and the unorganised or chemical ferments, bodies such as pepsin, which in infinitesimal amount produce changes in a considerable quantity of the substance acted upon, without themselves undergoing alteration.

It is better to reserve the term fermentation for the changes brought about by the organised ferments or living organisms, and to call the unorganised ferments enzymes, and the

changes which they produce zymolysis. Many enzymes are secreted by micro-organisms—for example, the liquefaction of gelatin is due to an enzyme, and if a little of the liquefied gelatin free from organisms be added to fresh solid gelatin it will produce liquefaction of this, and the same occurs in the presence of chloroform, which inhibits bacterial action. Yeast also secretes enzymes; it is unable to directly ferment cane sugar with the production of alcohol, but by the action of the enzyme ‘invertin’ the cane sugar becomes inverted, *i.e.* the molecule is split up into dextrose and lævulose, which are then further acted upon with the production of alcohol.

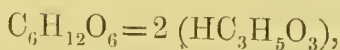
The following are the chief varieties of fermentation :

The Alcoholic Fermentation.—This is mainly brought about by the decomposition of sugars of the glucose group ($C_6H_{12}O_6$)_n by yeasts into alcohol and carbonic acid, but some of the bacteria and moulds also produce appreciable quantities of alcohol. It is not necessary, however, that glucose should be present, for other carbohydrates by the action of enzymes secreted by the organisms are converted into glucose, which is then fermented. The general reaction is as follows :



As a matter of fact a small amount of by-products appears in addition to the alcohol and carbonic acid, *viz.* glycerin, succinic acid, and higher alcohols. Until lately no enzyme had been obtained which would carry out this change; it only occurred when the living yeast-cells were present. Recently, however, an enzyme has been separated which will decompose glucose with the formation of alcohol.

The Lactic Acid Fermentation.—This is brought about chiefly by bacteria. Glucoses are converted into lactic acid, the reaction being :



but it is probably not actually so simple as this, for carbonic

acid is given off at the same time. A familiar example of this form of fermentation is the souring of milk.

The Butyric Acid Fermentation.—Butyric acid is formed from carbohydrates by the action of bacteria, mainly the *bacillus butyricus* and *clostridium butyricum*, the latter being an anaërobic organism, some by-products being formed in addition. Milk which has been just boiled usually undergoes the butyric rather than the lactic fermentation, the spores of the butyric organisms surviving.

The Acetic Acid Fermentation.—The conversion of alcohol into acetic acid is also due to bacteria, familiar examples of which are the souring of beer and wine. These are the more important fermentations brought about by micro-organisms, but there is no reason except conventional usage why pigment formation, nitrification, &c. should not be described as fermentations. It is uncertain whether the fermentive changes are brought about directly by the vital activities of the micro-organisms, or whether enzymes are first secreted. In a number of instances certainly the latter is the case, and the following are further examples of it :

1. Peptonising zymolysis, *e.g.* the liquefaction of gelatin.
2. Invert zymolysis.
3. Diastatic zymolysis.
4. 'Fermentation' of cellulose in the digestive tract—probably by secreted enzymes.
5. Curdling of milk by rennet-like enzymes.
6. 'Fermentation' of urea by an enzyme secreted by the *micrococcus ureæ*, with the formation of ammonium carbonate. These enzymes do not seem to possess any poisonous action.

Formation of Pigment.—Numerous organisms during their growth produce various coloured pigments, especially the bacteria of air and water. They are termed chromogenic bacteria, examples of which are the *sarcina lutea* and *micrococcus cereus flavus*, which form citron-yellow pigments; the *bacillus prodigiosus* and *spirillum rubrum*, red pigments;

the *bacillus violaceus*, a rich violet one; and the *bacillus pyocyaneus*, a blue. A large number of chromogenic organisms require oxygen for the production of the pigment, and potato is often a most favourable culture medium. In some cases the medium may become coloured, and the property of fluorescence be conferred upon it, as is the case with the *bacillus fluorescens liquefaciens*.

Phosphorescence or light-producing properties are developed by some bacteria, notably by many marine forms, and well seen in decomposing fish. The cholera spirillum is also stated to produce phosphorescence occasionally.

A *Necrotic action* on the tissues is produced by many pathogenic organisms. For example, the tubercle and glanders bacilli cause necrosis and caseation of the surrounding tissues.

Gas Production.—This is common to many organisms. It may consist of carbonic acid, hydrogen, or marsh gas, and in some cases of foul-smelling sulphur compounds, mercaptans, &c.

Basic or Alkaloidal Bodies: Ptomines.—These are a very important group of nitrogenous bodies, analogous to the vegetable alkaloids and mostly solid and crystalline in nature, which are formed by the action of bacteria on proteid and albuminoid matter. They often occur naturally in decomposing and putrefying food, meat, fish, &c., and, as many of them are virulent poisons, they are of considerable practical importance. Generally speaking, the poisoning due to tainted food is from the absorption of toxic ptomines formed by bacterial action. A number of toxic ptomines were isolated by Brieger from cultivations of pathogenic microbes, and great importance was once attached to them. They are referred to in the chapters describing the pathogenic organisms.

Brieger's work, however, needs revision, for the methods he employed for their isolation were not such as to exclude alteration by the reagents employed.

Stevenson obtained traces of a highly poisonous crystalline ptomine from some sardines which caused death. Vaughan has isolated a body, tyrotoxicon, apparently identical with diazobenzene, from poisonous cheese and milk. Mytilotoxin ($C_6H_{15}NO_2$) is the specific poison of toxic mussels. The ptomines are also of considerable medico-legal importance.

Toxic proteids are also formed by bacteria. Martin and others have described them as allied to the proteoses. Roux and Yersin considered that the diphtheria poison might be an enzyme, while Brieger and Fränkel regard it as albuminous. These non-basic chemical poisons were named tox-albumins, and were believed to be the specific toxic poisons of the pathogenic bacteria. The latest researches, however, seem to show that they are not proteid, and in the present state of our knowledge it will be better, perhaps, to term them simply 'toxins.'

They will be referred to in the sections dealing with the pathogenic organisms.

Recognising that many micro-organisms bear a causal relation to disease, several explanations have been advanced to account for the production of disease symptoms and death, such, for example, as (1) by the deoxidation of the blood, (2) by the accumulation of the organisms to such an extent as to block the blood-vessels and interfere with the functions of organs, (3) by the consumption of the proteids of the body, (4) by the destruction of the blood-corpuscles, (5) by the poisonous actions of the micro-organisms. The latter is now regarded as the true explanation, and the poisonous substances or toxins are probably secretions of the bacteria, or in some cases, perhaps, integral parts of the bacterial cells.

LITERATURE.

On Nitrification, see Warington : *Jour. Chem. Soc.* 1886, *et seq.*, Frankland, *Cantor Lectures*, 1892, and *Nature*, 1890, *et seq.* *On Bacterial Products*, '*Ptomaines and Leucomaines*,' by VAUGHAN AND NOVY, 1896 (*Bibliog.*), '*Ueber Ptomaine*,' by BRIEGER, 1885. For General Bibliography see Hamilton's *Text Book of Pathology*, ii. part 2.

CHAPTER II.

METHODS OF CULTIVATING AND ISOLATING ORGANISMS.

It is necessary for the satisfactory study of micro-organisms in their relation to the various processes of infection and disease, of fermentation, putrefaction and the like, to isolate them in a state of purity, that is, to separate the different species in a mixture, and, having done so, to be able to cultivate, grow, or propagate them on suitable soils through successive generations. A slight consideration will show that unless we work with pure cultures, that is, cultures consisting of a single species, we can never be sure that a particular result is due to a given organism; in a mixture some or all of the organisms may conduce to the effect produced. With regard to the pathogenic organisms, or disease germs, Koch has laid down the following conditions, which have been termed Koch's Postulates, which must be complied with before the relation of an organism to a disease process can be said to be completely demonstrated:

1. The organism in question must be met with in the tissues, fluids, or organs of the animal affected with, or dead from, the disease.
2. The organism must be isolated and cultivated outside the body on suitable media for successive generations.
3. The isolated and cultivated organism on inoculation into a suitable animal should reproduce the disease.
4. In the inoculated animal the same organism must be found.

It is true that in some instances, as in leprosy, one or other of these conditions may be wanting, and the dependence of the disease on a certain micro-organism cannot be said to be on such a satisfactory basis as in other diseases, though from various considerations there may be little doubt that such is the case.

In order to isolate organisms in a state of purity it is absolutely necessary to employ vessels, instruments, and culture media which are sterile, that is, free from any living organisms, and to possess the means of manipulating them in such a way that the entrance of organisms from without is prevented, and contamination avoided. Various methods of destroying and of getting rid of organisms are known, such as the use of chemical 'germicides,' heat, and filtration through porous porcelain. The addition of chemical germicides, such as carbolic acid or corrosive sublimate, is out of the question, for although the vessels and media might be rendered sterile thereby, the growth of the organisms which are being investigated would be equally prevented, so that the last two, viz. heat and filtration, are those which are employed, the former being used for vessels, instruments, and culture media, solid and fluid, the latter for fluid culture media only.

Various apparatus are needed for sterilisation and the preparation of culture media. These will now be described.

Hot-air Oven (fig. 1).—This is a somewhat cubical box of sheet iron with double walls, having an air-space of nearly an inch between them, and furnished with a door. The bottom should be protected with a loose piece of sheet iron which can be renewed as it 'burns' away. The top is perforated with a couple of holes, through one of which a chemical thermometer, registering to 200° C., is inserted in a cork, while through the other some form of mercurial regulator can be introduced if required, but is not usually needed. In the hot-air steriliser all thin glass vessels and

cotton-wool are sterilised by heating to a temperature of about 140°C . by means of a Bunsen burner or small gas-stove under the oven, which is supported on a suitable iron stand.

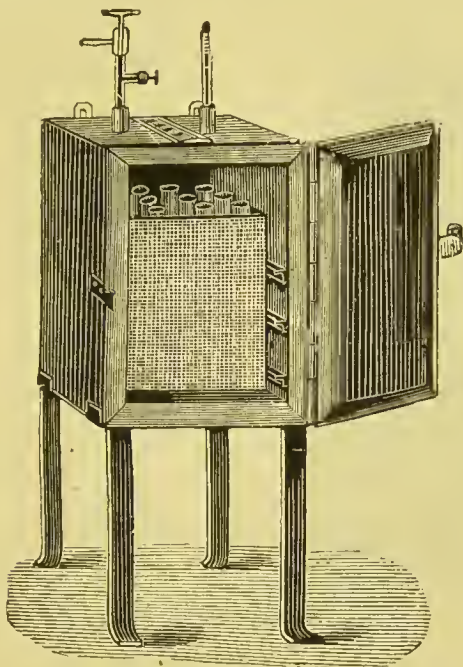


FIG. 1.—HOT-AIR OVEN.

If the oven is placed on a table or other wooden support a piece of sheet iron or asbestos cardboard should be laid over the wood to protect it from the heat.

It is evident that an inexpensive substitute for the hot-air oven may be readily devised, any iron box or even a biscuit-tin being used for the purpose.

Steam Steriliser (fig. 2).—This consists of a cylindrical or rectangular vessel of 'tin' or copper, covered on the outside with a layer of felt or asbestos, having a false perforated bottom supported a few inches above the true bottom, and provided with a movable lid. In the steam steriliser or 'steamer' the culture media, and thick glass vessels and

other apparatus which would crack or be damaged by the high temperature of the hot-air oven, are sterilised by steam.



FIG. 2.—STEAM STERILISER.

The lower chamber of the steamer, below the false bottom, is partly filled with water, which is boiled by means of a Bunsen burner. Above the false bottom the culture media or apparatus are placed, and are sterilised by the steam at 100°C . which fills this space.

Here again an inexpensive substitute may be devised; the ordinary kitchen saucepan with steamer will do well for many purposes, while a 'warren pot' answers admirably.

Autoclave (fig. 3).—This is most useful for many purposes, but it is expensive and not a necessity, for the steam steriliser can be made to answer almost every purpose for which the autoclave is employed with the expenditure of a little more time and trouble. It consists of a strong boiler of brass or gun-metal with a movable lid, which can be fixed down by means of screw-bolts. The lid is provided with a safety valve, a gauge for indicating the pressure and temperature, and a stop-cock to relieve the pressure if required. A small quantity of water is placed in the bottom, and the media or apparatus to be sterilised having been introduced, the lid is screwed down. It is heated by means of one or more Bunsen burners, which are turned down when the required temperature has been reached. The temperature usually employed is about 115° to 125°C . Care should be taken when heating fluids that the vessels are not

too full and that the autoclave is allowed to cool down to below 100°C . before relieving the pressure by opening the stop-cock, or a good deal may be lost by violent ebullition.

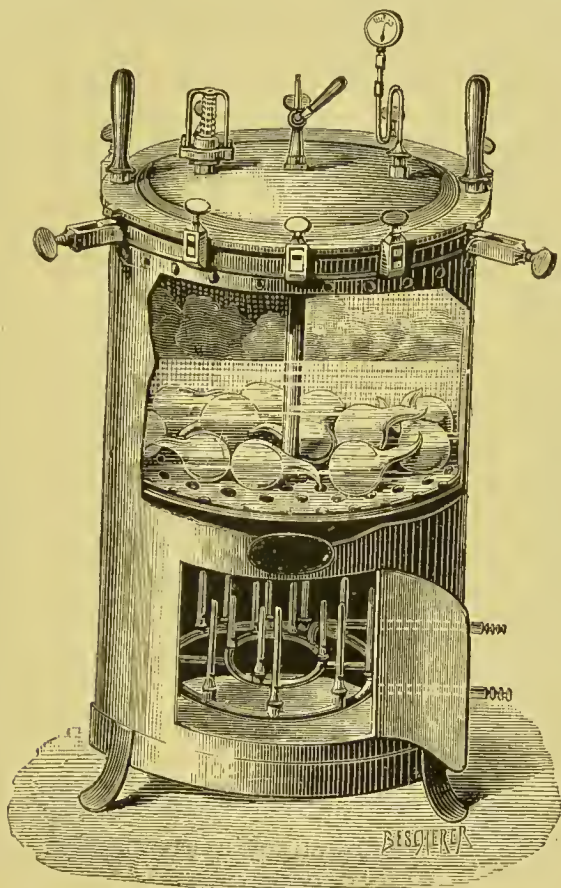


FIG. 3.—AUTOCLOVE.

Air-pump.—An exhaust pump is very useful for many purposes, as evaporating to dryness *in vacuo*, filtration through porous porcelain filters, &c. Any form will do, but of the more elaborate ones the Fleuss pump (fig. 4) made by the Pulsometer Engineering Company is by far the best. In using it care must be taken that no fluid or moisture gains

access to the barrel; to avoid this it is a good plan to intercept the connecting pipe with a vessel containing strong sulphuric acid (D, fig. 4), over the *surface* of which the exhausted air has to pass. A double-necked Woulfe's bottle does well for this, the inlet and outlet tubes going down nearly to, but not dipping below, the surface of the sulphuric acid.

For greasing the vessels, &c. to make air-tight joints beeswax dissolved in the Fleuss pump oil with the aid of heat to a stiff paste is a good composition.

Bell-jars with ground rims and one or two tubules are useful for evaporation *in vacuo*. They should stand on a square of thick ground glass. To make an air-tight joint the surface of the rim of the bell-jar should be well greased and pushed thoroughly home on the ground-glass plate. A thick ridge of grease should then be plastered all round the angle formed by the rim of the bell-jar and the glass plate. Thick rubber pressure tubing must be used for connections, and joints be well greased. For evaporating large quantities of fluid the writer devised a copper stand with shelves, the shelves supporting glass dishes containing alternately strong sulphuric acid and the fluid to be evaporated, the whole being placed under a suitable bell-jar. A mercurial gauge is a useful addition to show the amount of exhaust and the occurrence of leakage. The ordinary glass filter pumps used in chemical work and worked by a stream of water are also useful for many purposes.

Porous Porcelain Filters.—The two forms which are generally employed are the Pasteur-Chamberland and the Berkefeld. These consist of 'candles' composed in the former of unglazed porous porcelain, in the latter of a specially prepared diatomaceous earth. The filtration through the Pasteur-Chamberland is much slower than through the Berkefeld. Both give a germ-free filtrate, but the latter should be employed if the fluid is thick or contains many

particles; a preliminary filtration through paper is an advantage. A useful method of conducting filtration is the following. The filter 'candle' B (fig. 4) is connected by a short length of pressure tubing with a piece of glass tubing passing through a rubber cork in the neck of an ordinary filtering flask C. The 'candle' is placed in a jar A, such as a glass measure or urine-jar, which is filled up with the solution to be filtered. The lateral branch of the filter flask is then connected with the air-pump. On

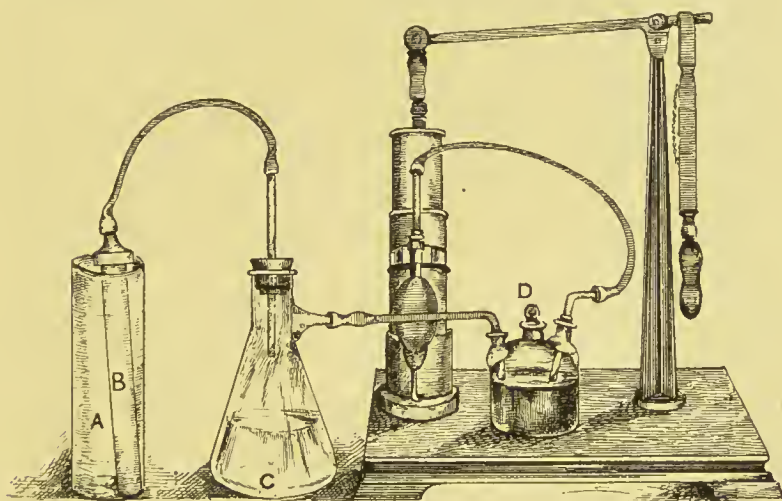


FIG. 4.—FLEUSS EXHAUST PUMP, ARRANGED FOR FILTRATION.

exhausting, the fluid passes through the filter 'candle' over into the filtering flask, in which it is collected. Before use the 'candle' should be well scrubbed and some water or half per cent. carbolic run through to clean it, or the whole may be sterilised in the steamer for an hour or two. After use the same process should be gone through to cleanse it.

Flasks, Beakers, and Test-tubes.—A good supply of these are required of various sizes: Erlenmeyer and ordinary shapes, tall and short form of beakers, &c. A few yeast flasks are also useful. These consist of an ordinary small flask, into

the neck of which a piece of glass tubing is sealed at right angles, and turned down so that it extends nearly as low as the bottom of the flask (fig. 12).

The best size of test-tube is $6'' \times \frac{5}{8}''$; a few $6'' \times \frac{1}{2}''$ should also be kept.

Platinum Needles (fig. 5).—Two or three platinum needles ('öse') are required. They consist of about two inches of platinum wire in a handle of glass rod. One end of the glass rod is softened in the Bunsen or blowpipe flame, and about an eighth of an inch of the platinum wire is embedded in it with a forceps, the wire having been first heated to a red



FIG. 5.—PLATINUM NEEDLES.

heat. The glass-wire joint is then well annealed in the flame and allowed to cool slowly. Two thicknesses of platinum wire are desirable, viz. 0.4 mm. for most purposes, but a thicker wire about 0.7 mm. where stiffness is required, and one or two 3 inches or more in length are useful.

Forceps, Needles, &c.—Several forceps are necessary, the ordinary dissecting form in two or three sizes, one or two pairs of fine pointed, and two or three small brass ones. A few ordinary sewing needles of various sizes mounted in wooden handles serve all purposes.

Glass Pipettes and Capillary Tubes.—These are useful for preserving or storing blood or pus, &c. for examination, for sterile water in making cover-glass specimens, and for many other purposes. A piece of glass tubing is heated in the blowpipe flame until quite soft; it is then *taken out of the flame* and the two ends pulled steadily apart; this forms a capillary tube of greater or lesser length and larger or smaller diameter, and it can be sealed off in con-

venient lengths. To make a pipette, proceed in the same way: seal off the capillary tube two or three inches from the wide tube, then heat this close up to where it was heated



FIG. 6.—GLASS PIPETTE.

before, and draw out again and seal off two or three inches from the bulb. In this way a capillary tube with a wide bulb at its middle is formed (fig. 6).

India-rubber Caps.—A few india-rubber caps for capping test-tube or flask cultures are required. They retard evaporation and the desiccation of the medium, and prevent the entrance of moulds. For use they should be soaked in 1–500 corrosive sublimate solution. Tinfoil may also be used to cover the tops of tubes and flasks.

PREPARATION OF STERILE TEST-TUBES, FLASKS, ETC. FOR THE RECEPTION OR MANIPULATION OF CULTURE MEDIA.

Glass Vessels.—Thoroughly wash the vessels in soap-and-water, and rinse with tap-water: then rinse with 25 per cent. hydrochloric acid. Afterwards wash well with tap-water and drain. A final rinse with distilled water is an advantage, as no deposit then occurs on drying. Place the cleansed vessels in the hot-air oven, which should be cold to start with, and raise the temperature to about 150° C. The high temperature should be maintained for half an hour, when the gas is turned out and the oven allowed to slowly cool. When the tubes, flasks, &c. are removed from the oven they must at once be plugged with a firm plug of sterilised cotton-wool. They are now ready to receive the culture media, &c.

To Sterilise Cotton-wool.—The best quality non-absorbent cotton-wool should be used for plugging purposes. Pull the wool well apart so as to assist the penetration of heat; in the

compressed condition the interior is difficult to sterilise. Place the separated wool in the hot-air oven and slowly raise the temperature to 130°C ., and keep it at this for at least an hour. Above 130°C . cotton-wool becomes brown and brittle.

If tubes or flasks are required in a hurry they may be rapidly sterilised as follows. After washing in water, they are rinsed with alcohol and then with ether. They are then dried by carefully warming over the flame of a Bunsen burner, holding in a suitable forceps or holder, the ether evaporating and burning at the mouth. When dry, a pledget of cotton-wool is held in the forceps and singed in the flame, and, while burning, the tube or flask is plugged with it. The tube or flask is then well flamed in the Bunsen burner, so that it is far too hot to handle, and allowed to cool.

Petri's dishes for plate cultures are cleaned and sterilised as described for tubes and flasks.

When thick glass vessels, such as measures, &c. have to be sterilised it is not safe to carry it out in the hot-air steriliser, as they are very liable to crack. After cleaning, they should be plugged with sterilised cotton-wool and steamed in the steam steriliser for an hour on two successive days.

CULTURE MEDIA.

Acid Beef-broth.—The basis of the most important culture media, viz. peptone beef-broth, gelatin, and agar-agar, is an infusion of meat prepared usually from beef. In order to prepare this infusion, which may be termed acid beef-broth, we proceed as follows: Take 1 lb. of beef ('rump steak') free from fat, chop fine or mince, add 1 litre of tap-water, and allow it to simmer in a saucepan for one hour, cool, remove any solidified fat from the surface, and filter through filter-paper into a clean glass flask. If not required for immediate use, plug the neck of the flask with cotton-wool and steam in

the steam steriliser (or boil) for three-quarters of an hour on two successive days; it may then be kept indefinitely.

Peptone Beef-broth.—Take 1 litre of the acid beef-broth, add to this 10 grams of peptone and 5 grams of common salt (i.e. 1 per cent. peptone and 0·5 per cent. sodium chloride), mix in a flask, and steam in the steam steriliser until dissolved. When dissolved, remove from the steam steriliser and render slightly alkaline with a 10 per cent. solution of sodium carbonate, *glazed* litmus-paper being used as an indicator. Having done this, return to the steamer for one hour, then filter through two thicknesses of German filter-paper (No. 597). It should now be quite clear and bright and may be kept in bulk, after steaming for three-quarters of an hour on two successive days, or the sterilised test-tubes may be filled with it to the depth of an inch or an inch and a half. The test-tubes after filling are steamed for half an hour on two successive days. Beef-broth if prepared in this manner should need no clarifying, but if it should filter at all cloudy, cool, add the white of an egg beaten up with the shell, and steam for half an hour, filter, and finally sterilise as before.

Glycerin Beef-broth is prepared in the same manner, 4–6 per cent. of glycerin being added to the fluid after filtration.

Peptone Water.—Add to distilled water 1 per cent. of peptone and $\frac{1}{2}$ per cent. of common salt, dissolve by heat, make faintly alkaline with sodium carbonate, steam for one hour and filter. Fill sterile test-tubes to the depth of an inch and a half and sterilise for half an hour in the steamer on two successive days.

For the cholera vibrio it is an advantage to add 1 per cent. instead of $\frac{1}{2}$ per cent. of common salt (Dunham's solution).

Beer-wort.—Procure beer-wort (preferably unhopped) from the brewery. Allow it to stand in a cool place for twelve hours, filter, and then steam for an hour and filter

again. Fill sterile test-tubes and sterilise in the steamer for half an hour on two successive days.

Veal-broth.—For some purposes veal presents advantages over beef, as for growing the tubercle bacillus. When obtained from the butcher's the veal is usually powdered with flour; this should be brushed and washed off as far as possible, as it renders the broth turbid and difficult to clarify.

The veal-broth is made in precisely the same way as peptone beef-broth. It is often, however, slightly alkaline, so that it does not require so much sodium carbonate to be added to it. It generally requires clarifying with the white of an egg as described for peptone beef-broth. For the tubercle bacillus about 4 to 6 per cent. of glycerin should be added in addition.

Grape-sugar Broth.—For the cultivation of anaërobic organisms the addition of 2 per cent. of grape-sugar is an advantage. It should be added after filtration.

Nutrient Gelatin.—Take 1 litre of the acid beef-broth in a large beaker and add to it 100 grams of the best gold label gelatin, 10 grams of peptone, and 5 grams of common salt. Place in the steamer until quite dissolved. Then render faintly alkaline, as for the peptone beef-broth; cool to 60°C., and add the white of an egg, stir well, and return to the steamer for one hour. Filter through two thicknesses of filter-paper in a hot-water funnel (this is best, but it may be done in the steamer at a low temperature, i.e. 35°C.). Fill test-tubes with the filtrate to the depth of an inch and a half and then steam for ten minutes on three successive days. After the third steaming the tubes are allowed to solidify, either in the upright or oblique position, according as they are required for stab or surface cultivation.

In hot summer weather 15 or even 20 per cent. of gelatin (150 grams or 200 grams to the litre) are necessary for the product to remain solid. Nutrient gelatin melts at 25° C. or a little under. Prolonged boiling diminishes and

ultimately destroys the gelatinising power of gelatin, so the less heating it has the better. It must not be autoclaved.

Grape-sugar Gelatin.—Ordinary gelatin with the addition of 2 per cent. of grape-sugar.

Beer-wort Gelatin.—This is the best culture medium for yeasts and some of the fungi (e.g. ringworm). Procure from the brewery some beer-wort, preferably unhopped, and add to every litre 100 grams of gelatin. Dissolve, clarify, and filter, as in the case of ordinary gelatin. It is not neutralised.

Nutrient Agar-agar.—This is one of our most valuable culture media, and has the advantage over nutrient gelatin that it remains solid at blood-heat.

The bar agar is superior to the stick or powder form ; it should be well washed by soaking in water for half an hour, and all the water is then squeezed from it by wringing it in a towel. Add 15 grams (i.e. $1\frac{1}{2}$ per cent.) of the washed agar to 1 litre of acid beef-broth, together with 10 grams of peptone and 5 grams of common salt in a large glass flask, place in the steamer until dissolved (half an hour to one hour), and then render alkaline as for peptone beef-broth ; allow it to cool to 60° C., and add the white of an egg. Place back in the steamer for an hour and a half, then filter through an *agar filter-paper* (now specially made) in a hot-water funnel or in the steamer. By this treatment a litre of agar should pass through the filter in two to three hours. If it does not come through clear, add another white of egg and repeat the process.

If an autoclave is available, a quicker and better method is, after neutralising and adding the white of an egg, to place in the autoclave with a small beaker inverted over the mouth of the flask, and heat to 134° C. (two atmospheres pressure) for half an hour. Turn the gas out, and allow to cool without opening the stop-cock. When cool, open, and filter through the *special agar filter-paper* in a hot-water funnel ; the agar will

pass through in about ten minutes or a quarter of an hour. Fill test-tubes to the depth of an inch and a half and sterilise for half an hour in the steamer on two successive days. Solidify in the upright or oblique position as required.

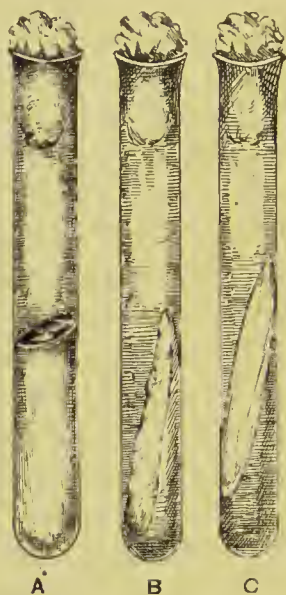


FIG. 7.—TUBES OF CULTURE MEDIA.

A, upright agar; B, potato;
C, sloping agar.

Another method of preparing agar-agar is to first steep the agar in 1 per cent. acetic acid for a quarter of an hour, then to drain and wash it so as to thoroughly remove the acid. The further procedure is the same as detailed above. This yields a very clear, pale product, and is perhaps preferable when an autoclave is not available.

Glycerin Agar.—Add 4 to 6 per cent. of glycerin to the nutrient agar after filtration and proceed as before.

Grape-sugar Agar.—Add 2 per cent. of grape-sugar to the nutrient agar after filtration and proceed as before.

Litmus Agar.—The addition of neutral litmus to the various culture media is a useful method of demonstrating the production of acid or alkali by organisms. To prepare the litmus solution take the lump litmus, powder finely, and boil with distilled water so that a saturated solution is obtained. Filter, and preserve in a flask stoppered with cotton-wool, after sterilising by boiling for half an hour on two successive days.

Of this litmus infusion add sufficient to the nutrient agar, or, better, sugar agar, after filtration, to tinge it a distinct purplish colour. After steaming the colour has usually disappeared, but returns as the tubes cool.

Milk.—If possible, procure separated milk, but if unable

to do this, take ordinary new milk, place in a tall cylinder or beaker, and allow it to stand overnight in a cool place, preferably in an ice safe. Then pipette off the milk from the bottom, rejecting the cream. Introduce into sterilised test-tubes to the depth of about an inch to an inch and a half and steam for one hour on two successive days.

Litmus Milk.—Proceed in the same manner, but add sufficient of the litmus solution (see Litmus Agar) to render it a purplish colour.

Potatoes.—Choose sound potatoes, and scrub them well with water to remove dirt. Cut off the ends, and with a cork-borer, slightly smaller than the test-tubes which are used, bore through the potato so that a cylindrical piece is removed. Push this out of the borer, and divide it into two portions by a very oblique transverse cut, so that two wedge-shaped pieces are obtained, and in this manner prepare as many pieces as there are tubes to be filled. Place them in a basin under the tap, and allow the water to flow over them for about two hours. This prevents the darkening of the potatoes in the subsequent steaming. The test-tubes for the potato-wedges are prepared as follows: After plugging and sterilising in the ordinary way, introduce a small pledget of sterilised wool into each, push to the bottom, and moisten with a little sterilised distilled water. Drop the potato-wedges into the tubes, plug, and sterilise by steaming for three-quarters of an hour on two successive days. Roux's tubes (fig. 8) may also be used, the lower bulb being filled with water.

Blood Serum.—Clean some glass jars of about 2 to 3 litres capacity, plug with wool, and sterilise in the steamer for an hour on three successive days. Bleed a



FIG. 8.—ROUX'S
TUBE FOR
POTATO.

horse, with aseptic precautions, and catch the blood in these sterilised jars. Allow the jars to stand in a cool place for twelve hours. Then pipette off the clear serum with a sterile pipette, and fill the sterilised test-tubes to the depth of about an inch and a half. The tubes are then arranged in the sloping position on the shelves of the serum inspissator (Hueppe's), the temperature of which should be about 50° C. They remain at this temperature for thirty hours; then raise it to 65° C., at which temperature they will coagulate in about four to six hours and are now ready for use. It is well, however, to place them in the blood-heat incubator for a night, so that any contaminating bacteria may form colonies, and the contaminated tubes can then be rejected.

The serum inspissator is practically a large incubator (see p. 49) with inclined shelves, on which the tubes of serum rest.

Fluid Serum, &c.—Fluid blood serum, ascitic and hydrocele fluids, &c. are sometimes useful, and may be used alone or mixed with peptone beef-broth in various proportions.

Ascitic or hydrocele fluid may be readily obtained by using sterile trocars, &c. and carrying out the tapping with aseptic precautions, collecting the fluid in sterilised flasks. It is better to collect in several small flasks than in one large one.

Fluid blood serum may be obtained by collecting blood with aseptic precautions in sterilised flasks. When the blood has coagulated and the serum separated, the serum is pipetted off with a sterile pipette into sterile flasks.

The flasks of serum, &c. should be kept in a warm place for two or three days to make sure that they are sterile, those in which a growth appears being rejected.

Serum, ascitic fluid, &c. may also be obtained sterile by filtering through a sterilised Berkefeld filter into sterile flasks.

Löffler's Blood Serum.—Procure the serum in the manner described for the simple blood serum. To every three parts of serum add one part of sterile grape-sugar broth, mix, fill tubes, and inspissate as before.¹

Serum Agar (Kauthack and Stevens²).—Ascitic, pleuritic, or hydrocele fluid is collected in clean (not necessarily sterilised) flasks, and allowed to stand overnight in a cool place to allow the sediment or blood to collect. The clear fluid is then poured off, and to each litre enough of a 10 per cent. caustic potash solution is added to render it very distinctly alkaline—usually about 2 c.c. to every 100 c.c. of the fluid. The alkaline fluid is heated in the autoclave for two to four hours. To this fluid 1·5 to 2 per cent. of agar (finely cut and steeped in water) is added, and the mixture heated until the agar is dissolved. It is then filtered, introduced into test-tubes, sterilised, and solidified in the ordinary way. The addition of 5 per cent. of glycerin and 1 per cent. of glucose is an advantage.

Alkali Albumin (Lorrain-Smith³).—To 100 c.c. of fresh serum add 1 to 1·5 c.c. of a 10 per cent. caustic soda solution; mix, and introduce into test-tubes in the ordinary way. Place the test-tubes in the slanting position in the autoclave at 115° C. for twenty minutes, or in the steamer on three successive days.

Egg Cultures (Hueppe).—These are very useful for some purposes. A hen's egg is taken and one end sterilised by washing with carbonate of soda solution, rinsing in sterile water, soaking in 1–500 corrosive sublimate solution, and washing in alcohol and in ether. A small hole is then chipped in the shell with a sterile needle and the inoculation made through this. The hole is afterwards closed with a little sterilised wool and collodion.

¹ Löffler originally used ox-serum.

² *St. Barth. Hosp. Re.* :. xxxi. 1895, p. 89. (Also *Centr. f. Bakt.*)

³ *Brit. Med. Journ.* 1894, i. p. 1177.

Ushinsky's Fluid.

	Parts.
Sodium chloride	5-7
Calcium chloride	0.1
Magnesium sulphate	0.2-0.4
Di-potassium phosphate.	2-2.5
Ammonium lactate	6-7
Sodium asparaginate	3-4
Glycerin	30-40
Water	1,000

A solution of known composition which can be used for investigating the chemical products of bacteria. Pathogenic organisms grow well in it and produce their toxins.

Pasteur's Fluid.

	Parts.
Cane-sugar	10
Tartrate of ammonia	1
The ash of 1 gramme of yeast	—
Water	100

A good culture fluid for yeasts, &c.

THE CULTIVATION AND ISOLATION OF MICRO-ORGANISMS.

It must be clearly understood that micro-organisms cannot be identified by their microscopical characters alone. We can state from a microscopical examination the form of an organism, that it is a bacillus, or a micrococcus, or a sarcina, and that it is motile or non-motile, but beyond this we cannot as a rule go. It is necessary in most cases to ascertain the characters of the growth of organisms on the various culture media before a name can be given to them, and this is the main reason for having a varied assortment of nutrient soils. It is likewise necessary for the successful cultivation of pathogenic organisms, i.e. those connected with disease processes and developing in or upon the bodies of man and animals, to keep

the cultures at a temperature approximating to that of the host. For this purpose some form of incubator is required. This consists of a box or chamber of copper or iron with double walls (fig. 9), the space between which is filled with water, the outside being covered with wood or felt, or some other

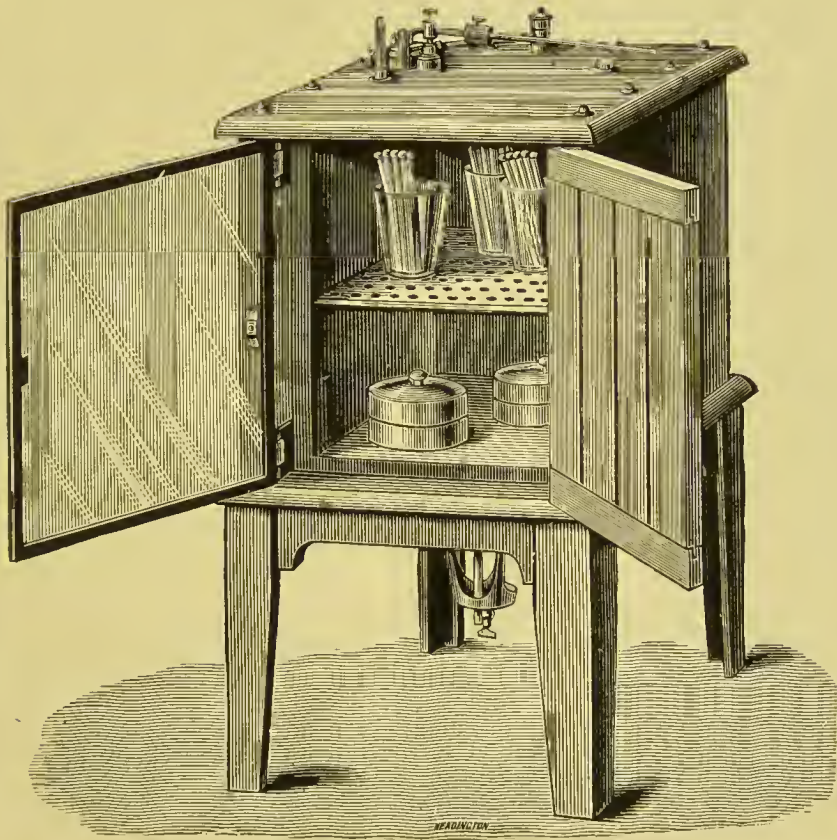


FIG. 9.—HEARSON'S INCUBATOR

non-conductor. The water between the walls is heated by means of a small burner, the gas supply for which has to pass through some form of regulator inserted in the water, so that the temperature, indicated by a thermometer inserted through a hole in the top, can be kept constant.

The regulator is usually a mercurial one, such as Page's or Reichert's, the principle of their action being that as the temperature rises the mercury expands and at a certain point cuts off the greater part of the gas-supply, only sufficient gas then passing to keep the flame of the burner alight. This point can be varied either by a sliding tube, in Page's, or by a screw, in Reichert's, so that the temperature may be set at any desired point. In Hearson's incubator, which is one of the best forms, the regulator consists of a capsule containing a fluid of a certain boiling point, which when ebullition takes place raises a lever and so cuts off the gas. While the Hearson regulator is a very constant one, it has the disadvantage that it can only be used for a range of temperature of a few degrees unless the capsule be changed. At least one incubator is required, but two or three are an advantage. If there be only one, the regulator should be set for a temperature of 37° C.; if more, another should be kept at 22° C., and the third at 30° C. The incubator at 37° C. is termed the warm or blood-heat, and that at 22° C. the cool or room temperature one. An ordinary warm room will answer most of the purposes of the cool incubator.

A substitute for the large and expensive incubator can be readily devised. An ordinary chemical hot-water oven can be employed, or simply a smaller tin set in a somewhat larger one, the interspace being filled with water; while, with a little scheming, regulators can be dispensed with by making use of a small gas or lamp flame, varying its size and distance from the bottom until the right temperature has been attained. Gas is certainly a great convenience, but where none is available regulating oil lamps can be obtained to take its place.

Gelatin will remain solid only at temperatures below 22° C., and cannot therefore be placed in the blood-heat incubator without becoming for practical purposes a fluid medium. Agar, however—and this is one of its most

valuable properties—does not melt much under the temperature of boiling water, though when melted it does not set again until the temperature has fallen to about 45° C. Gelatin is therefore usually reserved for use at low temperatures, while agar, blood serum, potato, and the fluid media can be used either at low or high temperatures indifferently. Agar is often a better cultivating medium than gelatin, even at low temperatures, probably because it is so much moister. The growths in fluid media are not usually particularly characteristic; a general turbidity forms, but sometimes an organism produces a film on the surface which another similar organism does not, thus affording a distinction. Not only do the characters of the growths of organisms on media differ more or less, but in some instances chemical changes occur in the media which afford valuable information in the differentiation of species. Thus many organisms exert a peptonising effect on gelatin, and render it fluid sooner or later, while others have no such action. Milk is coagulated by some organisms, the coagulation being brought about in one of two ways, either by the production of acids and precipitation of the caseinogen, or by the action of a rennet-like ferment with the formation of a clot of casein. All organisms which liquefy gelatin coagulate milk, but the converse is not the case. Agar is carbohydrate, not albuminoid, in nature, and is not liquefied by any organism. In fluid media, such as broth and peptone water, chemical tests can be applied, especially for indol, which is formed by some organisms but not by others.

Method of Inoculating Tubes.—Supposing there is a pure culture in a test-tube from which subcultures are desired, the following is the method of procedure. Having selected some tubes of sterile media, gelatin, agar, broth, &c. they are placed in a test-tube rack. Their mouths are then singed by holding in the Bunsen flame for a few seconds and with a forceps, also sterilised by heating in the flame, the wool plugs are loosened by a rotatory motion, and then partially withdrawn.

A suitable platinum needle is selected and carefully straightened. The original culture-tube having been singed and its plug partially withdrawn, in the same manner as the sterile tubes, is then taken in the left hand between the thumb and index finger with the palm upwards, and is held obliquely, the mouth of the tube pointing to the right, a tube of sterile medium being held side by side with the original culture in an exactly similar manner. The wire end of the platinum needle is then heated to redness by holding nearly vertically in the flame, and the lower part of the glass rod is also heated carefully. Holding the sterilised needle between the finger and thumb of the right hand, the plug of the original culture is now withdrawn by grasping between the ring and little fingers of the right hand, and is held there while the platinum needle is carefully introduced into the tube without touching the mouth or sides, and a trace of the growth is picked up with it, preferably from the margin. To be sure that the needle is cool it may be first touched on the medium where there is no growth. The needle is quickly withdrawn without touching the sides of the tube and the plug at once replaced. The plug of the sterile tube is now withdrawn in the same manner, and the inoculated needle introduced. If a surface culture is desired, a single light streak is made with the needle from the bottom to the top of the medium without penetrating the surface; if a stab culture, the needle is plunged steadily into the centre of the medium and withdrawn; if a fluid one, the growth removed is rubbed up on the side of the tube at the margin of the fluid, and the emulsion washed down by tilting the tube. The inoculation having been completed, the plug is quickly replaced, and the needle is again heated in the flame to destroy the remains of the growth upon it. If the original culture is in a fluid medium a looped platinum needle may sometimes be used with advantage. The inoculations completed, the mouths of the tubes are singed and the wool plugs pushed in level with

the lip. Before replacing the plugs they may, if desired, for greater safety, be taken with the forceps, held in the flame for a second or two, and pushed while burning into the tube, and this procedure must always be adopted if the plug be dropped or brushes against anything. If the tubes have to be kept for any time, especially in the blood-heat incubator, they should be capped with the rubber caps which have been soaked in 1-500 corrosive sublimate solution.

Anaërobic Cultures.—Many organisms refuse to grow in the presence of oxygen, and various expedients have to be adopted to exclude it. The simplest of all is to make the cultivation in a deep stab in sugar-agar or gelatin. Narrow test-tubes filled three parts full with the medium are best, and immediately before the inoculation they should be placed upright in a beaker of water, boiled for five minutes, and then cooled and solidified in cold water. The object of this is to soften the medium so that it does not split, as a dry medium will, when the needle is plunged into it, while the needle track closes up, and the dissolved oxygen is got rid of. The tubes being cool, the inoculation is made with a long, thin needle, preferably straight, but in some cases a closed loop will pick up more material. It is inoculated and plunged steadily into the centre of the medium, nearly to the bottom, and then withdrawn, and the wool plug replaced and singed. The tube, held in a cloth, is then carefully heated at the upper border of the medium so as to melt this slightly and seal the puncture, and a well-fitting rubber cap is applied while the tube is hot. The heating expels a portion of the air and, with a well-fitting cap, creates a negative pressure within the tube, so that the residual oxygen is not so readily absorbed. The tubes are placed in the incubator at a suitable temperature, and it will be found that the most strictly anaërobic organisms can be cultivated in this way.

When, however, an organism is required to grow anaërobically on the surface of the medium, or in a fluid medium, some

other method must be adopted. One is to place the tubes under the receiver of an air pump and exhaust as completely as possible. This is not very convenient, for it is difficult without great care to keep a vacuum, and special receivers must be used when the cultures have to be incubated at blood-heat, while with fluid media ebullition causes considerable difficulty.

A simpler and far preferable method is to absorb the oxygen by means of alkaline pyrogallic acid, and so cultivate in an atmosphere of nitrogen. This can be carried out in two ways—either in a wide-mouth bottle with well-fitting



FIG. 10.—BUCHNER'S
TUBE ARRANGED FOR
ANAEROBIC CULTIVA-
TION.

glass stopper, sufficiently large to contain the test-tubes, or in Buchner's tubes. For the first, the stopper of the bottle is vaselined and the inoculated test-tubes are placed in it. Some solution of pyrogallic acid in water is run into the bottle by means of a thistle funnel, and then some caustic potash solution. As quickly as possible the thistle funnel is taken out, without mixing the solutions, and the stopper inserted and twisted well home, some melted paraffin poured all round the joint and melted in with a hot iron. The solutions in the bottle may now be well mixed, and the whole placed in a suitable incubator.

Buchner's tubes (fig. 10) are convenient for single test-tube cultures. They consist of a large, strong glass tube, sealed at the bottom, large enough to take a test-tube, and having a constriction about an inch and a half from the bottom. The constriction supports the test-tube culture, while the mixture of pyrogallic acid and caustic potash fill

the portion below the constriction. A well-fitting rubber cork closes the mouth of the tube, and the joint may be paraffined for additional security. A strong solution of pyrogalllic acid is employed, and mixed with an equal volume of 20 per cent. caustic potash in the bulb.

Lastly, the displacement of the atmosphere by means of hydrogen may be adopted, and is the best method for fluid cultures. Hydrogen does not seem to inhibit the growth of any anaerobic organisms, whereas carbon dioxide gas, which might be still more conveniently used, has a very decided inhibitory action on some species. The hydrogen is best generated from zinc and sulphuric acid in a Kipp apparatus, or the compressed gas in cylinders may be used. Care must be taken that all joints are tight, and they may be paraffined with advantage. The gas should be passed through a strong solution of caustic potash, and may be passed through some alkaline pyrogalllic acid if the most rigorous condition of anaerobiosis is desired, but for ordinary purposes this is not essential; it also passes through two or three fairly firm plugs of cotton-wool to remove organisms; these must be dry, for if moist they may stop the passage of the gas altogether.

For tube cultures Fränkel's method may be adopted (fig. 11). The broth or gelatin is introduced into a large and strong test-tube, the mouth of which is plugged with a rubber cork pierced with two holes. Through these holes two pieces of glass tubing pass, one to the bottom of the tube, the other just through the

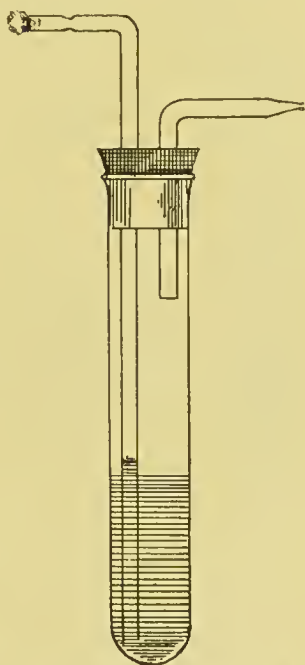


FIG. 11.—FRÄNKEL'S TUBE FOR ANAEROBIC CULTIVATION.

cork. Outside the cork these tubes are bent over at right angles and are each drawn out slightly so as to contract their lumen at about their middle. The long tube is connected with the hydrogen supply and a current of the gas is passed through and escapes by the shorter tube. After the gas has been passing for twenty minutes to half an hour, and *all oxygen having been expelled*, the distal, i.e. shorter, tube is sealed off at the contracted portion in the Bunsen or blowpipe flame, and then the proximal or longer one in the same manner. The rubber cork must, of course, fit well, and the joints should be paraffined. If gelatin be the medium, it must be kept fluid in a bath of warm water while the hydrogen is passing.

For broth cultures, which are essential for examining toxic products and for inoculating animals for the preparation of antitoxins, flasks are used which are fitted with an india-rubber cork with two holes. Through the holes two pieces of glass tubing pass, one to the bottom of the flask, the other just through the cork. The ends of these tubes are plugged with cotton-wool, and the whole—flask, cork, tubes, and medium—is sterilised. The medium is inoculated from a recent culture by momentarily removing the cork. Hydrogen is then passed through from a Kipp apparatus, the long tube being connected with the hydrogen supply. After passing for about half an hour, the tubes are sealed off, and the flask incubated. For convenience of sealing the tubes should be drawn out slightly.

As many organisms produce gas during their growth, it may be necessary to have some means of allowing its escape, or the flasks may burst owing to the pressure. This can be done by adjusting a mercury valve, and may be carried out in a simple manner by a method devised by the writer. 'Yeast flasks,' which can be obtained in various sizes, are made use of, and are filled three parts full with a 2 per cent. grape-sugar bouillon. The neck is corked with a perforated

rubber cork (A, fig. 12), through which a glass tube, B, passes to the bottom of the flask, projecting two inches above the rubber cork and here plugged with cotton-wool. The lateral tube of the yeast flask is also plugged with cotton-wool, care being taken that the plugs are loose enough to allow air to pass freely. The whole is sterilised and inoculated. The

glass tube, B, which passes through the rubber cork is then connected with a Kipp's or other hydrogen-generating apparatus by means of a rubber tube, and a current of hydrogen is passed through the flask. The hydrogen bubbles through the bouillon and escapes by the lateral tube. After the gas has been passing for about an hour a small tube containing mercury, C, is applied to the end of the lateral branch, so that the open end just dips below the surface of the mercury, and the tube, B, which passes through the rubber cork

is sealed off in the blowpipe flame, care being taken that all the air has been expelled from the flask by a free current of hydrogen. The flask, with the capsule of mercury applied to the end of the lateral branch, can then be placed in the incubator. Thus the mercury forms a valve through which air cannot enter, while gases formed by the growth of the organism have free exit.

Plate Cultivations.—The method of plate culture is one of

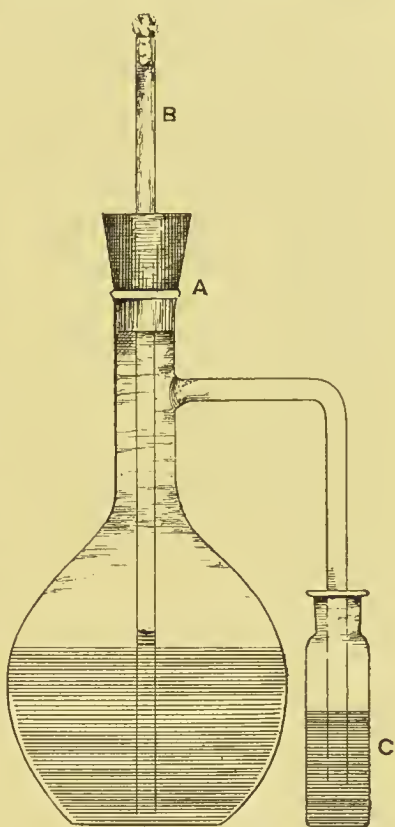


FIG. 12.—YEAST FLASK ARRANGED FOR ANAEROBIC CULTIVATION.

the most important in bacteriology. It is used for three purposes: (1) for obtaining pure cultivations, i.e. cultures containing a single species, from a mixture of organisms; (2) for the enumeration of organisms; and (3) for ascertaining the characters of the colonies of organisms as an aid to their diagnosis.

Before the introduction of plate cultivations pure cultures of organisms could only be obtained by chance, or by the dilution method, which also was by no means certain. The dilution method consisted in estimating approximately the number of organisms in a given volume of fluid by means of an instrument on the same principle as the hæmatocytometer. The fluid is then diluted by the addition of more sterile fluid so that a given volume of the dilution will contain a single organism, assuming that the organisms are evenly distributed throughout the fluid. By transferring this volume to tubes of sterile media, pure cultivations will in some cases be obtained, for a single organism only has been sown.

An example will better explain this method. Suppose that each c.c. of the original fluid contains 100 organisms, by the addition of 99 c.c. of sterile fluid to each c.c. the dilution will contain, if the organisms are evenly distributed, one organism in each c.c.; therefore, by transferring 1 c.c. of the dilution to sterile media, in some instances, at any rate, a single organism only will be sown and a pure cultivation be obtained. It is obvious, however, that this method is at best an uncertain one, but the plate-culture method to a large extent obviates this uncertainty. It depends upon the following principles. In gelatin and agar we have media which are fluid above 40° C. and solid at lower temperatures. By inoculating the fluid gelatin or agar; thoroughly mixing, and then pouring on to a level sterilised surface, so that the medium solidifies in a thin film, the organisms, wherever they may be situated, are fixed and are unable to wander, and, being in

a good nutrient soil, grow and multiply and ultimately form a visible growth or colony; and each colony having arisen from a single organism, the growth is pure, and pure cultures can be obtained by inoculating tubes of sterile media from them.

Where suitable, sterile nutrient gelatin is usually employed for the preparation of plate cultivations, as it is more easily manipulated than agar. Three tubes of sterile nutrient gelatin are melted at a low temperature in a beaker of water (gelatin melts at 25°C .; the temperature should not exceed about 45°C .). The tubes may be termed respectively 1, 2, and 3. Tube No. 1 is inoculated, by means of a platinum needle, with a trace of the growth from which pure cultivations are desired. The trace of growth is thoroughly mixed up and distributed throughout the melted gelatin; but if this mixture were poured on to a sterile level surface so many organisms would be present in the film that the colonies which developed would not be separate, but would form a confluent growth. To obviate this difficulty a second and a third dilution are prepared. The second dilution is prepared by inoculating the tube of melted gelatin No. 2 with two to four small platinum loopfuls from tube No. 1, and thoroughly mixing up; and to be quite sure that the resulting colonies will be isolated from each other, a third dilution is prepared in the same manner by inoculating the tube of melted gelatin No. 3 with two to four platinum loopfuls from tube No. 2. In each case, the organisms having been distributed throughout the gelatin by rolling and gentle shaking, the wool plug is withdrawn from the mouth of the tube, and the mouth of the tube singed in the Bunsen burner to prevent contamination, then allowed to cool, and the melted gelatin poured on to a level sterile glass surface. Formerly plates of glass about six inches by four inches, sterilised in iron boxes in the hot-air steriliser and placed on a levelling stand, were used (hence the name); but now shallow glass dishes with lids, about three or four inches in diameter, known as Petri dishes (fig. 13), are almost always

employed. They are sterilised in the hot-air steriliser and used cold; the melted gelatin being poured in, the dish is tilted to diffuse the gelatin over the bottom of the dish,

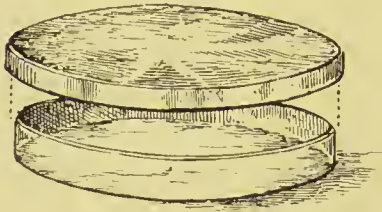


FIG. 13.—PETRI DISH FOR PLATE CULTIVATION.

placed on a level table for the gelatin to set, and then stored in the cool incubator. The plates are examined every day with a hand lens, if necessary, or with a low power of the microscope, the dish being turned bottom upwards on the stage of the microscope for this

purpose. When the colonies have developed, inoculations can be made from them by means of a platinum needle on to tubes of sterile media. The colonies having arisen from single organisms are pure, and the resulting subcultures are also pure. Different species of organisms usually form colonies having different appearances, so that the colonies are an aid in diagnosis and enable the different species to be picked out from a mixture. Whereas the plate cultivation prepared from tube No. 1 is usually of little use, plates 2 or 3, or both, can be made use of, and it is apparent that, to make certain of isolating all the organisms from a mixture, several sets of plates should be prepared.

In addition to the isolation of species from mixtures and for diagnosis, plate cultures are also used to enumerate organisms. Assuming that the colonies arise from a single organism, which is approximately the case, the number of colonies represents the number of organisms originally introduced into the gelatin, and if a known weight or volume of the material inoculated be used, the number of organisms in it can be calculated. For example, in the bacteriological examination of water a measured volume of the water is added to each tube of melted gelatin by means of a sterilised pipette, and by counting the resulting colonies the number

of organisms originally present in 1 c.c. of the water can be estimated.

Agar plate cultures can be prepared in a similar way. The agar, however, must be brought to a temperature of nearly boiling before it melts; it is then allowed to cool to 45° or 40° C. and the tubes inoculated in the same manner as for a gelatin plate culture described above. Unless the manipulations be carried out expeditiously the agar will set, or the agar film in the Petri dish be lumpy.

It is often a good plan to incubate plates bottom upwards in order to get rid of the condensation water.

The plate-culture method can be modified to suit particular circumstances; for example, the melted gelatin or agar, uninoculated, may be poured into the dishes and allowed to set, and the film then inoculated by streaking or painting with the material, or by pouring a few drops of broth containing the organisms upon it. This is practically the only way in which blood serum can be used, the sterile blood serum being placed in the Petri dish, solidified in the inspissator in the same manner as blood-serum tubes, and the coagulated film inoculated.

Esmarch's Roll Cultures.—Another modification of the plate-culture method is known as Esmarch's roll culture. For this purpose large test-tubes ('boiling tubes'), at least an inch in diameter and six inches long, are sterilised and plugged with cotton-wool. The sterile melted gelatin, about 10 c.c., is poured in and inoculated, the wool plug replaced, and the tube held in the horizontal position and rotated under a stream of cold water until the gelatin has set. In this way the gelatin forms a thin film over the inside of the tube, but a little practice is required to get it evenly distributed. The colonies then develop in the film of gelatin, which is quite analogous to a film in a Petri dish.

Anaërobic Plate Cultivations are sometimes required. The plate cultures after preparation as described above can be

placed in the receiver of an air-pump which is exhausted, and the colonies develop *in vacuo*, or the film in the Petri dish may be covered with a piece of sterilised mica well pressed down, or with a layer of sterilised oil, or pure liquid paraffin. In Botkin's method a bell-jar standing in a glass dish is made use of. The Petri dishes are placed on a support within the bell-jar, and mercury or oil is poured into the glass dish. By means of a piece of bent glass tubing a stream of hydrogen is passed into the bell-jar under its rim so as to displace the air, which bubbles out through the oil or mercury. When the air has been entirely displaced the glass tube is removed, the bell-jar weighted, and the whole placed in the incubator.

The Esmarch roll cultures are a very convenient form for anaërobic plate cultures. The wool plug is replaced by a rubber cork with two holes in it, through which inlet and outlet glass tubes pass, as in Fränkel's anaërobic tubes (fig. 11). The roll culture having been prepared, and the film set, hydrogen is passed in and the tubes sealed off, or, better still, the hydrogen is allowed to bubble through the inoculated melted gelatin, the test-tube meanwhile being kept in a little warm water to prevent the gelatin from setting, the tube sealed off, and the roll culture then prepared.

The addition of $\frac{1}{2}$ to 1 per cent. of sodium formate to the culture media much simplifies anaërobic cultivation; the tetanus bacillus for example can be grown in formate broth in a stoppered bottle without any elaborate precautions for excluding the last traces of air. The sodium formate should be added just previous to the last sterilisation, not before, or decomposition may occur.

CHAPTER III.

THE PREPARATION OF TISSUES AND ORGANISMS FOR STAINING
AND MOUNTING. STAINING AND STAINING METHODS.

A VERY large number of methods for preparing and staining tissues, bacteria, &c. have been published, but only a selected few which are generally useful will be given here. Any special methods available only in a single instance will be found at the respective places where they are required.

PREPARATION OF TISSUES.

As the demonstration of the bacteria in the tissues is of the primary importance the elaborate methods which have been described for fixing the tissue elements are not necessary in bacteriology. In all instances the tissues should be as fresh as possible; and it must be remembered that within a few hours of death they are invaded by numerous bacteria, from the air or from the cavities of the body, which may mask the original bacterial infection and lead to serious errors if this source of fallacy be not carefully borne in mind. In all cases the tissue should be cut into pieces of convenient size, not more than about a quarter of an inch in thickness, and organs if kept *en masse* should be sliced. Having been thus prepared, they may be treated by one of the following methods:

(a) Place directly in methylated spirit¹ for a week or fort-

¹ Methylated spirit free from mineral naphtha should be used, and can be obtained in quantities of five gallons, 'for scientific purposes only,' by special order from the Inland Revenue Authorities, Somerset House, W.C. If it cannot be procured, absolute alcohol must be employed.

night, and preserve until wanted in dilute methylated spirit (spirit 2 parts, water 1 part).

(b) Place in methylated spirit 1 part, water 2 parts, for twenty-four to forty-eight hours, transfer to methylated spirit and water, equal parts, and then to methylated spirit for like periods, and preserve in the dilute methylated spirit.

(c) Place in rectified spirit (86 per cent. alcohol) containing 1 per cent. of corrosive sublimate for twelve to forty-eight hours, and then preserve in the dilute methylated spirit.

(d) Place for six to twelve hours in a saturated aqueous solution of corrosive sublimate. This is prepared by saturating boiling distilled water with the corrosive sublimate, cooling and filtering. Keep in the dark. When removed from the corrosive sublimate solution the tissues must be washed in a stream of running water for an hour, or, better, placed in 75 per cent. alcohol deeply coloured with tincture of iodine, to remove the excess of corrosive sublimate and prevent precipitation. They are then preserved in the dilute methylated spirit, which should be changed once or twice. A precipitate of small black spherical masses is apt to form in tissues which have been treated with corrosive sublimate.

(e) Formalin, a 40 per cent. aqueous solution of formic aldehyde, has recently been introduced as a fixing agent and seems to act well. A solution of 1 part of formalin and 9 parts of water may be used, the pieces of tissue remaining in this for twelve to twenty-four hours. They are then washed in running water for an hour or two and preserved in the dilute methylated spirit.

All tissues after fixing should go for a day or two into methylated spirit, and should then be preserved in dilute methylated spirit—water 1 part, methylated spirit 2 parts.

The methods (c), (d), and (e) are to be recommended, especially the last two, as the tissue elements are well fixed thereby. In all cases the fixing fluid should be used in considerable excess. Fixing fluids containing potassium bichromate (as in

Müller's fluid) and chromic acid seem to prevent the bacteria from staining with any certainty, and should be avoided.

SECTION CUTTING.

In order to demonstrate bacteria in tissues satisfactorily, and their relation to the tissue elements, it is usually necessary to prepare sections. For this purpose either the freezing or paraffin method should be employed.

(a) *Freezing Method.*—The tissue, in suitable pieces, must first be soaked in water to remove the alcohol. A convenient way of doing this is to place the material in a wide-mouthed bottle, into the mouth of which an ordinary glass funnel is then introduced, and the bottle and the funnel are placed under a running stream of water. The water flowing through the funnel fills the bottle and overflows through the chinks between the neck of the bottle and the sides of the funnel; the pieces of tissue, being too large to pass, are retained in the bottle, and are thoroughly washed in the stream of water. With running water in this way the alcohol will be completely removed in from one to two hours; in still water, which should be changed two or three times, this result may not be attained for several hours, during which time there is an ever-increasing risk of bacterial contamination from without. *It is essential to remove all the alcohol, or the tissue will not freeze.*

When the alcohol has been removed, which is known by the tissue *sinking* in the water (lung is an exception—it always floats unless solid from any cause), the pieces are transferred to a strong mucilage of gum-acacia:

Gum-acacia	5 grams
Cane sugar	1 gram
Water	100 c.c.

Add a piece of thymol or a little carbolic acid to prevent

decomposition. Hamilton saturates the solution with boric acid, which does very well.

In this gum solution they remain for twelve to forty-eight hours, according to their size and the time at the disposal of the investigator, and are then cut on one of the numerous ether-freezing microtomes now to be obtained, such as Swift's (fig. 14) or Cathcart's. The material must not be frozen so

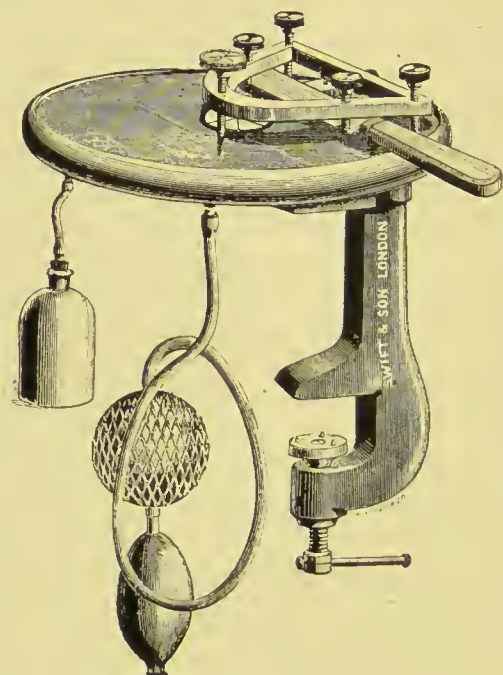


FIG. 14.—SWIFT'S ETHER-FREEZING MICROTOME.

hard that the sections roll up and fall off the knife; the sugar in the above solution should prevent this. When cut the sections are transferred to two or three lots of distilled water, preferably slightly warmed, to remove the gum, and can then be stained, or preserved in equal parts of methylated spirit and water until wanted.

Bacteria seem to retain their staining properties better in the tissue in bulk than when it has been cut up into

sections. Although the bacteria may stain well in sections for some time after preparation, it frequently happens that in a month or two they refuse to stain. Such is certainly the case with anthrax tissues, but not with tubercle or leprosy, the bacilli in sections of the latter seeming to retain their staining powers unaffected for an unlimited time.

(b) *Paraffin Method*.—Nothing can surpass the paraffin method for the thinness and beauty of the sections obtainable by it, and for some friable tissues, such as actinomycosis, it is almost essential. The tissue, in suitable pieces for cutting, is transferred from the methylated spirit preservative solution to absolute alcohol—which may have to be changed once unless a fairly large volume is employed—for twenty-four to forty-eight hours. It is then taken out, lightly compressed between the folds of a *dry* cloth or piece of blotting-paper to remove the superfluous alcohol, and placed in an excess of xylol. In this it remains for eighteen to twenty-four hours until cleared. This is recognised by the material assuming a more or less semi-transparent condition, and the process may be much accelerated by warming the xylol to from 37° to 50° C. in the blood-heat incubator or paraffin oven, the bottle containing the xylol being well stoppered. When cleared it is ready to go into the bath of melted paraffin. A paraffin of a fairly high melting point is perhaps the best, viz. 45° to 55° C., and is placed in glass capsules in an oven which can be kept uniformly heated to the required temperature. An ordinary chemical hot-water oven does admirably for this purpose, and is heated by a small special form of Bunsen burner with mica chimney, the temperature being regulated by some form of mercurial regulator which is set a degree or two above the melting point of the paraffin employed. The tissue is taken out of the xylol, blotted to remove the excess, and placed in the melted paraffin for six to twelve hours. It is then embedded by pouring a little of the melted paraffin into a watch-glass, or into a small box formed of folded paper

or lead-foil, or by bringing together two L-shaped pieces of brass on a glass plate so that a rectangular cavity is produced. Still more convenient are the porcelain pans used for moist colours. The pieces of tissue are then taken out with a small warmed forceps or needle, adjusted to the position they are wanted to occupy, and more melted paraffin poured in, so as to cover them. When a film of solid paraffin has formed, the whole arrangement is carefully placed in cold water so as to cool it rapidly.

A new paraffin is frequently crystalline in structure, and acts much better after it has been kept melted for some weeks, or is much improved by heating nearly to its boiling point for five or six days (P. T. Beale). The xylol for clearing may be used several times and the paraffin repeatedly, the remains of old tissues being removed.

Other clearing agents, such as chloroform, turpentine, and cedar oil, may be used instead of xylol. The paraffin method is usually straightforward, but *small* pieces of tissue must not be left too long either in absolute alcohol or in the paraffin bath, for they are apt to become too hard to cut. Thyroid tissue is also rather troublesome; it becomes very hard unless the whole process is carried out as rapidly as possible.

In order to prepare sections from material imbedded in paraffin some form of microtome must be employed. An ether-freezing microtome *can* be employed with a little manipulation, the paraffin block being placed in a little melted paraffin on the freezing plate so that it is cemented there, and sections cut with the razor or plane iron, as though it had been frozen (it is *not* to be frozen). It is better, however, to use some special form of microtome, the Cambridge 'Rocker' (fig. 15), or a modification of it, or the Minot, being, perhaps, the best. The block of paraffin containing the tissue is trimmed with a knife to remove the excess, and is cemented to the carrier of the microtome with a little melted paraffin,

or by melting the paraffin on it with a hot iron (end of a file, &c.) or a match. The union may be made more secure by melting the paraffin around the base of the block with a hot iron.

Having fixed the paraffin block to the carrier, sections may then be cut of any degree of thinness. In order to do this it is essential for the knife or razor to have a keen edge and one of the right nature, for a knife may be perfectly sharp and yet the sections as they are cut roll up in such a manner that it is difficult to flatten them. Though this may be due to the paraffin not having the right consistence, owing to cold

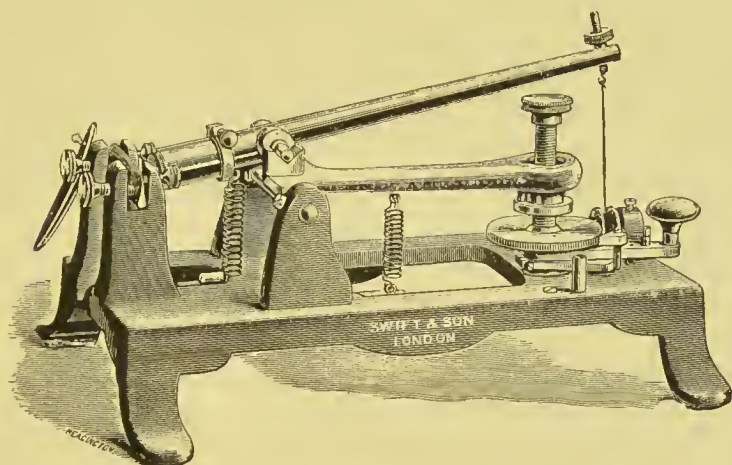


FIG. 15.—CAMBRIDGE ROCKING MICROTOME.

weather or some other factor, in the majority of instances it is the edge of the knife which is at fault. Provided the knife be sharp, stropping on the palm of the hand will usually remedy this difficulty. The paraffin being of the right consistence, and the knife in good order, the sections as they are cut should be flat and should adhere together at adjacent margins so that a ribbon of greater or shorter length is formed. Satisfactory sections having been obtained, they are dealt with in the following manner.

A little distilled water is placed on a clean slide and the sections as cut, as many as convenient, are picked up with a needle or camel's-hair brush, and transferred to the slide so that they float on the water. The slide is then warmed gently by holding it some distance above a Bunsen or spirit lamp flame, so as to *soften* but not to *melt* the paraffin. This causes the sections to become perfectly flat. Fixing the section at the corner with a needle, the water is tilted off the slide, and the slides with the sections lying on them are set up on edge to dry in a warm place—the blood-heat incubator answers admirably. Instead of warming over the flame, the slides with the floating sections may be placed on the top of the paraffin oven for a minute or two, or in the blood-heat incubator for ten to fifteen minutes. The slides can be manipulated in an hour or two if dried in the incubator, but it is best to allow them to dry all night. It will be found after this treatment that the sections adhere sufficiently firmly to the slides for all the ordinary methods of staining to be carried out without disarranging them, which would be fatal. The sections must be fairly thin, however; if they are at all thick they do not adhere nearly so well.

Supposing that while cutting, the sections, in spite of all precautions, curl up instead of lying flat, it is still often possible to obtain a few which can be mounted. They may sometimes be unrolled by cautious manipulation with a couple of needles after having been softened by warming, or a needle or knife-blade may be held close to the edge of the microtome knife during cutting, so that the sections instead of curling slide up against this guide.

Tissues embedded in paraffin may be kept indefinitely in labelled pill-boxes and cut all at once or from time to time as required. The slides also, with the sections sticking to them, can be kept until it is convenient to stain, if preserved free from dust, as in a slide box.

COVER-GLASS SPECIMENS.

The satisfactory preparation of cover-glass specimens is one of the most important in bacteriology, for they are used for the examination of cultivations of bacteria, and of blood or other fluids or secretions, organs, &c. for the presence of micro-organisms.

It is necessary in the first place to have clean cover-glasses of the right kind; they must be thin, otherwise the higher powers cannot be employed to examine the preparations, and those described as 'No. 1' should be purchased, ' $\frac{3}{4}$ -inch squares' being a very convenient size. These serve not only for cover-glass specimens, but also for covering sections; it is well also to have a few of the same thickness but larger, viz. $\frac{7}{8}$ -inch or 1 inch squares, for large sections. In order to clean them they should be gently boiled in a porcelain dish with 10 per cent. carbonate of soda solution for a few minutes; they are then well washed, and are next treated with strong sulphuric acid, warmed carefully in a porcelain dish, for a few minutes. The acid having been poured off, they are again rinsed in several changes of water, and are then preserved in a stoppered glass pot in absolute alcohol.

One of these clean cover-glasses is taken and dried with a clean soft linen rag or handkerchief, and placed flat on a convenient support on the work-table—a white glazed tile is excellent—with the corner projecting so that it can be conveniently picked up with the forceps. A droplet (i.e. *small* drop) of water is then placed on it, in the middle, by means of a platinum needle, the end of which has been bent so as to form a small loop, or with a small glass pipette (fig. 6). Theoretically, sterilised distilled water should be used, a few cubic centimetres of distilled water being boiled in a sterilised and plugged test-tube for two or three minutes and cooled; but ordinary distilled water (or even tap water) may usually be employed. A thin film of organisms has now to be formed on the cover-glass, and

the following is the method of procedure with a culture on a solid medium such as agar or gelatin. The cap, if there is one, being removed from the tube, the mouth of the tube is singed in the Bunsen flame. With a pair of forceps, also heated in the flame, the wool plug is partially withdrawn. The tube is then held between the thumb and forefinger of the left hand, in an oblique position, the palm being upwards, and the mouth of the tube somewhat higher than the sealed end and pointing towards the ulnar side. A straight platinum needle is then taken with the thumb and forefinger of the right hand and sterilised by holding nearly vertically in the Bunsen flame and heating to a red-heat, the lower portion of the glass rod being also carefully heated. The wool plug of the tube is then taken between the ring and little fingers of the right hand, removed, and held while with the platinum needle a trace of the growth is removed from the tube. The needle should have cooled before this is done, or, if a thick one, it may be rapidly cooled by touching the medium in the tube *by the side of the growth* before removing any. The plug is immediately replaced, but if it has been dropped or placed on the table it must be held in the forceps and singed in the flame before returning to the tube. When done with, the mouth of the tube is singed in the flame and the cap replaced if necessary.

A mere trace of the growth from a culture should be taken, just sufficient to soil the tip of the platinum needle, or the preparation will be too crowded, and this is rubbed up well with the droplet of water on the cover-glass, so as to form an emulsion, which is then spread over the surface. As a general rule the material should be well rubbed up, but in some instances this is inadvisable, as a particular formation or characteristic grouping might be disturbed thereby, in which case, after a slight admixture with the water, the emulsion is gently spread. Having spread the emulsion, the cover-glass is allowed to dry, or may be dried by gentle

warming over the Bunsen flame, in which case it is well to hold the preparation in the fingers, and move backwards and forwards over the flame. Having dried the film, it has next to be fixed, which is accomplished by holding the cover-glass, film side up, in the forceps and passing pretty rapidly three times through the Bunsen flame. The object of this 'fixing' is to thoroughly dry the film and coagulate albuminous material, whereby the film adheres better to the glass, and is not so likely to be washed off in the subsequent processes of staining and washing, &c. Fixing may also tend to diminish the staining capacity of the extraneous matter mixed with the organisms. The preparations are now ready for staining.

When the culture is in a fluid medium, such as broth, the tube is manipulated in the same way, the deposit at the bottom having been shaken up if necessary, and a loopful of the fluid removed with a looped platinum needle, transferred to the cover-glass, spread out, dried, and fixed as before, but as the medium is fluid there is usually no need to add a droplet of water.

If a cover-glass specimen of blood or pus is required the procedure is much the same. A little of the material is taken up with a looped platinum needle and spread in a thin film over the cover-glass, which is then dried and fixed. If necessary, a droplet of distilled water or normal salt solution may be used to dilute the material so as to obtain a thinner film. If a cover-glass specimen is to be made from an organ, a particle of the pulp is picked up and an emulsion made as before, or a small piece of the organ may be held in sterile forceps and the cut surface gently smeared over the cover-glass, which is then dried and fixed; these are termed 'smear preparations.'

To get the best results it is preferable, however, to submit films of blood or pus or smear preparations to the action of some chemical fixing agent. The following is a simple and

efficient method :—Draw a drop of blood from a prick, and pick it up by touching a cover-glass; spread by applying another cover-glass, but avoid all pressure. Separate covers by sliding from each other, and immerse without drying in the following solution :

Absolute alcohol saturated with eosin	25 c.c.
Pure ether	25 c.c.
Alcoholic solution of corrosive sublimate (2 grams in 10 c.c.)	5 drops

For four cover-glasses 5 to 10 c.c. of this solution are required, and they should remain in it three to four minutes (it may be prolonged for hours without harm); they are then removed with a forceps and well rinsed in water; stain for not more than a minute in a saturated aqueous solution of methylene blue, wash quickly, dehydrate in absolute alcohol, clear in xylol, and mount in xylol balsam. The solution may be used for fixing blood, pus, sputum, &c. if the stain be omitted, and the preparations may then be stained or otherwise treated in any desired manner.¹

Cover-glass films may also be well fixed by treating them after drying with a mixture of equal parts of absolute alcohol and ether for five to fifteen minutes.

Impression Specimens.—These are employed to examine and preserve permanently the colonies or growth of organisms so that their characteristic formation may be observed. In plate cultivations this is very simple. A clean cover-glass is sterilised in the flame and, having cooled, is cautiously lowered on to a selected colony with a sterile needle, avoiding all lateral movement. It is gently pressed on to the colony and then carefully raised by means of a couple of needles, and the colony should adhere to the glass, and may be dried and fixed. The colonies in gelatin tube cultures may also be used if the growth is removed from the tube. This can be done

¹ Gulland, *Brit. Med. Journ.* 1897, i. p. 65 .

by dipping the tube for a few seconds into hot water; the gelatin round the walls of the tube will be melted, and the gelatin can then be tilted out of the tube on to a glass plate or tile.

STAINS AND STAINING METHODS.

Micro-organisms being so minute and transparent, it is usually advisable to stain or dye them, so that they can be more readily examined. In some instances organisms have a particular staining reaction, which may serve as an aid to their identification. At the same time, when investigating any organism, examination in the fresh and living condition must never be omitted, for it is only thus that its motility and life history can be studied. Only general methods are given here; special ones will be found where they are to be employed.

1. Löffler's Alkaline Methylene Blue.

Concentrated alcoholic solution of methylene blue .	30 c.c.
Solution of caustic potash, 0·01 per cent.	100 c.c.

A very useful staining solution. Cultures should be quite fresh, or the organisms do not stain well. When the organisms are mixed with a good deal of other material, as in blood, or where there is much débris, this is one of the best staining solutions to employ.

Stain cover-glass specimens three to ten minutes, and sections half to twenty-four hours.

2. Carbol-Methylene Blue (Kühne).

Methylene blue	1·5 gram
Absolute alcohol	10 c.c.
Five per cent. aqueous solution of carbolic acid .	100 c.c.

A more intense staining solution than the former, and very useful for sections, which are stained for half to six hours.

3. Anilin Gentian Violet.

Saturated alcoholic solution of gentian violet .	30 c.c.
Anilin water	100 c.c.

The anilin water is prepared by shaking a few drops of anilin with distilled water, allowing the mixture to stand for a few minutes, and filtering. Squire recommends 3 c.c. of anilin and 90 c.c. of water as a good proportion.

This solution is a very useful general stain for cover-glasses, which are stained for two or three minutes. It is the solution made use of in Gram's method of staining.

4. Carbol-Fuchsin (Ziehl-Neelsen solution).

Fuchsin	1 part
Absolute alcohol	10 parts
Five per cent. aqueous solution of carbolic acid	100 parts

Dissolve the fuchsin in the absolute alcohol and then mix with the carbolic acid solution.

A very intense staining solution. Can be used for cover-glass specimens (one to three minutes) or sections (ten to thirty minutes). It is often best diluted with two to six parts of water for cover-glass specimens.

5. Carbol-Thionine Blue (Nicolle).

Saturated solution of Thionine in alcohol (90%)	10 c.c.
One per cent. aqueous solution of carbolic acid	100 c.c.

A stain now coming into general use. Sections can be stained in half to one minute. This solution may be used for a modified Gram's method (see below).

6. Chenzinsky's Solution.

Saturated aqueous solution of methylene blue	$2\frac{1}{2}$ parts
Half per cent. solution of eosin in 75% of alcohol	1 part
Water	2 parts

The solution should be freshly prepared. A good double stain for smear preparations, cover-glass preparations of blood or pus, &c. Stain for two to five minutes.

7. Eosin.

A somewhat diffuse stain. Is used for counter-staining the tissues in Gram's method, and for staining blood corpuscles and leucocytes.

A $\frac{1}{2}$ to 1 per cent. aqueous or alcoholic solution should be used, and the staining should not be prolonged for more than about half a minute.

8. Bismarck Brown.

A saturated aqueous solution should be prepared and diluted somewhat for use. A good counter-stain for the tissues in Gram's method. Stain for two to five minutes.

9. Orange-rubin.

Prepare saturated aqueous solutions of Orange G. and Rubin S. Mix equal volumes and dilute with water until of a light port wine colour. Stain tissues for five to fifteen minutes. A good contrast stain for tubercle and actinomycosis.

10. Picro-carmin.

This is best bought ready prepared. Sections are stained in the solution for half to one hour, washed, then placed in a watch-glass of spirit, to which three or four drops of hydrochloric acid have been added, for two or three minutes, then well washed in water. The section can now be counter-stained with Löffler's blue or by Gram's method.

11. Hæmatoxylin.

Ehrlich's formula is one of the best and can be obtained ready for use. It is a histological and not a bacterial stain. Sections are treated as follows :—

1. Distilled water, one to two minutes.

2. Stain with the hæmatoxylin solution for five to thirty minutes. In some cases the solution is preferably diluted somewhat with distilled water.

3. Rinse in distilled water.

4. Rinse in distilled water containing a *trace* of hydrochloric or acetic acid.

5. Treat with distilled water containing a *trace* of ammonia. The sections remain in this until they assume a deep blue colour.

6. They can then be dehydrated, cleared and mounted, or counter-stained with eosin or orange-rubin and then mounted.

Hæmatoxylin makes a good contrast stain for the tubercle and the leprosy bacillus and for actinomycosis.

12. Ehrlich-Biondi Triple Stain.

This is best bought ready for use. It is a good histological stain for tissues and leucocytes. Actinomycosis stains well by it.

Stain for ten to sixty minutes, then treat with methylated spirit until the material becomes greenish. Pass through absolute alcohol, clear, and mount.

13. Beale's Carmine.

Carmine (best)	10 grains
Liq. Ammon. Fort.	$\frac{1}{2}$ drachm
Pure glycerin	2 ounces
Alcohol	$\frac{1}{2}$ ounce
Distilled water	2 ounces

Used for staining *fresh* tissues and protozoa.

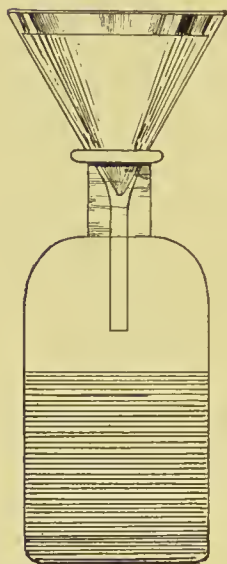


FIG. 16.—BOTTLE WITH STAINING SOLUTION.

Eosin, orange-rubin, hæmatoxylin, picrocarmine, and Beale's carmine keep well in solution; the remainder may or may not, and are best used fairly fresh. All stains should be filtered before use, and may be conveniently kept in bottles having a funnel fitted with a filter paper so that they are always ready (fig. 16).

Gram's Method.—This is one of the most beautiful and most satisfactory methods for staining micro-organisms. It is especially indicated for sections, specimens of blood, or smear or impression preparations, as the tissue or ground substance can be counter-stained so that the organisms show up in marked contrast. Ordinary cover-glass specimens do not usually require this method, unless débris or ground substance is present and the best result is desired. Unfortunately

Gram's method is not applicable to all organisms, for many do not retain their colour by the process. This disadvantage, however, is counterbalanced by the fact that it forms a valuable means of distinguishing organisms, and is always one of the points to be noted in bacteriological diagnosis. Most of the moulds, yeasts, streptothrix and sarcina forms, and cocci stain by it, though there are exceptions, but as regards the bacilli no rule can be laid down. Cover-glass specimens are stained for five to ten minutes, and sections for ten minutes to half an hour, in anilin gentian violet solution. Drain off the superfluous stain and then immerse in the following solution for one half to two minutes :

Iodine	1 part
Potassium iodide	2 parts
Distilled water	300 parts

The purple colour of the gentian violet changes to a dirty yellowish brown, and sections become much like a used tea-leaf. The specimens must not be passed on to the next solution until they have assumed the brown colour. Cover-glass specimens are best *immersed* in the solution *film side up*.

The specimens are removed from the iodine solution, drained, and then immersed in alcohol, preferably methylated spirit. In this the purple colour of the gentian violet returns and is dissolved out, so that they ultimately become colourless ; this is aided by moving them gently about, and for sections two or more baths of alcohol may be an advantage, a fresh one being substituted when the first has become deeply coloured. Cover-glass specimens decolourise much more readily than sections, and they should not be left longer than the point at which no more colour comes away, or the stain may be entirely removed. It is also important with cover-glass specimens to remember on which side of the glass the film is, for it is very difficult to ascertain this after

decolourising. After decolourising, cover-glass specimens are washed in water, dried, and mounted, or after washing the ground substance may be stained, if required, with eosin for a few seconds, or bismarck brown for two or three minutes, washed again in water, dried, and mounted. Sections after decolourising are passed through absolute alcohol and xylol before mounting, or, if required to be counter-stained, are immersed in eosin for half a minute, or bismarck brown for three to five minutes, and then passed through methylated spirit, absolute alcohol, and xylol.

Sections are frequently somewhat difficult to decolourise with alcohol alone, in which case it is often a good plan to treat them with a slightly acid alcohol (3 per cent. of hydrochloric acid) for a few seconds, and then return to the alcohol (Günther's method).

The iodine in Gram's method seems to act as a mordant, precipitating the stain in a relatively insoluble form in certain species of bacteria.

Weigert's Modification of Gram's Method.—In this process the sections, whether frozen or paraffin ones, should be stained on the slide. They are then stained with the anilin gentian violet and treated with the iodine solution as in the simple Gram's method. The iodine is then removed with filter-paper and the sections are flooded with anilin oil two or three times. This removes the colour and dehydrates. The anilin oil is removed by flooding two or three times with xylol.

Thionine may be used for Gram's method, the carbol solution being employed (No. 5 *ante*). Sections are stained for two to three minutes, then treated with an iodine solution somewhat stronger than Gram's, viz.:

Iodine	1 part
Potassium iodide	2 parts
Water	200 parts

The sections after remaining in this for one to two minutes

are decolourised in alcohol containing 1 per cent. of acetone (methylated spirit does very well), and subsequently treated as in Gram's method.

STAINING COVER-GLASS SPECIMENS.

For staining cover-glass, smear, and impression preparations, after fixing, the film is flooded with a drop or two of the solution, or a watch-glass is half filled with the solution and the preparation is floated on it, film side down, though if it should sink it makes little difference. Having stained sufficiently, the preparation is rinsed in water, first in a beaker or tumbler of tap-water, and then in one of distilled water. Having rinsed the superfluous colour away, the preparation is dried and mounted on a glass slide, film side down, in a drop of xylol balsam. In order to dry, having drained on to a piece of filter-paper for a second or two, remaining droplets of water are mopped up with a shred of filter-paper, and the preparation dried by holding in the fingers over the Bunsen flame and moving backwards and forwards. Or it may be allowed to dry spontaneously in the air, in which case it should always be set up on edge to drain, preferably on a ledge of filter-paper, which is folded into a sort of compressed **Z**— (z) shape. *The preparations must be completely dry before they are mounted in balsam.*

If there be much débris or other material which, when stained, would interfere with a clear view of the organisms, two or three expedients may be adopted. One is to stain for a short time with a solution which does not give a very dense colour, the best for this purpose being Löffler's methylene blue, or if the organism stains by it, Gram's method may be made use of, and will give the best result of any. Another plan is to treat the cover-glass specimen with acetic acid before staining; it may be just dipped in glacial acetic acid and immediately washed in distilled water, or may be immersed in a

watch-glass of 20 per cent. acetic acid for five to ten minutes, washed in distilled water, and then stained. A third is, after staining and washing, to rinse the preparation in dilute alcohol (methylated spirit 1 part, water 1 or 2 parts), and immediately wash again in water to stop the further action of the alcohol. If the film be thick, two or three rinses in the dilute alcohol may be necessary. This process gives excellent results with the sarcinæ, but the staining agent should be anilin gentian violet or carbol-fuchsin and not Löffler's blue unless it is allowed to act for fifteen to twenty minutes. The treatment with acetic acid before staining may be combined with decolourisation with alcohol after.

Preparations can always be examined in water after washing and before permanently mounting, in order to see whether they are satisfactory. A drop of water is placed on the slide, the specimen is mounted in this, film side down, and the upper surface of the cover-glass is dried with filter-paper. If satisfactory, it can be slipped off, dried, and mounted in balsam; or if not sufficiently deeply stained, or stained too deeply, it can be stained again, or further decolourised, as the case may be.

TREATMENT OF SECTIONS FOR STAINING AND MOUNTING.

(a) *Frozen Sections*.—If preserved in spirit they should be rinsed in distilled water before staining, unless the staining solution is an alcoholic one, in which case they may go straight into it. After staining they are well rinsed in water or methylated spirit to remove the excess of stain, and then have to be dehydrated and cleared before mounting. For dehydrating, if they have been washed in water, they should be well rinsed in methylated spirit¹ to remove the excess of

¹ Absolute alcohol may of course be employed instead of the first bath of methylated (or rectified) spirit, but methylated answers just as well and is less expensive.

water, and then transferred to absolute alcohol for a few seconds to two minutes, the time varying with the size and thickness of the section. In many cases—for instance where the anilin dyes have been used for staining—the sections must be passed as rapidly as possible *consistent with thorough dehydration* through the absolute alcohol to avoid removing too much of the colour. For clearing, xylol or cedar oil are the best agents, for they do not dissolve the anilin dyes; they will only clear, however, from absolute alcohol, hence the preliminary rinsing of water-washed sections with methylated spirit to prevent dilution of the subsequent bath of absolute alcohol. Oil of cloves can also be employed, but has the disadvantage that it dissolves the anilin dyes, and the colour of stained sections treated with it is apt to be less permanent; it has the advantage, however, of clearing out of methylated spirit, absolute alcohol being not necessary. The alcohol and clearing agents are conveniently placed in watch-glasses or small shallow glass capsules. The section is known to be cleared when it appears quite transparent and almost invisible when the watch-glass or capsule containing it is held over a dark surface. If it appears cloudy and opaque it is not properly cleared, which is due either to not having been in the clearing-agent a sufficiently long time, or to not having been properly dehydrated. If the section does not clear in two or three minutes it is evidently not sufficiently dehydrated, and should be returned to a fresh bath of absolute alcohol for a short time, and then transferred again to the clearing agent. Care should be taken that the watch-glasses used for the absolute alcohol and clearing agent are perfectly dry. The clearing agent, especially clove oil, can be used many times before becoming useless.

In transferring the sections from one solution to another an ordinary needle, fixed in a light wooden handle, is sufficient, or, better still, a piece of glass drawn out at one end, the section being carefully lifted by one corner to prevent

crumpling; but for the final process of mounting it is necessary to use a section lifter or cigarette-paper. The section having been carefully spread out, is raised up by means of the section lifter or cigarette-paper and transferred to the slide, any crinkles removed by spreading with a needle, the superfluous clearing agent drained off, a drop of xylol balsam put on, and it is then covered with a clean cover-glass. If clove-oil has been used as the clearing agent, after draining, the section should be blotted with two or three thicknesses of filter-paper to remove as much oil as possible before putting on the balsam. In blotting, firm pressure should be used, and the section will then adhere to the glass slide and not to the blotting-paper.

(b) *Paraffin Sections*.—The sections having been safely fixed on the slide, it is necessary in order to stain and mount to

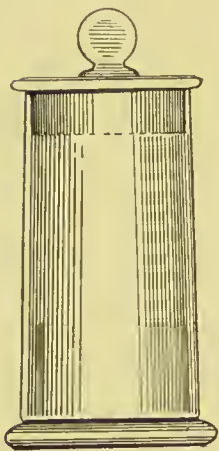


FIG. 17.—GLASS POT
FOR CLEARING, ETC.

remove the paraffin by solution in xylol. They are treated as follows: (1) immerse in xylol for one to two minutes; (2) absolute alcohol one to two minutes, to remove the xylol; (3) methylated spirit; (4) distilled water. They are now ready for staining, and are to be flooded with the staining solution or immersed in it, and after staining they are treated in the same manner, but in the reverse order, *i.e.* (1) distilled water; (2) methylated spirit; (3) absolute alcohol; (4) xylol. On being removed from the xylol they are drained for a few seconds, a drop of xylol balsam is then put on, and the section covered

with a clean cover-glass. Glass pots (fig. 17) filled with the alcohol, xylol, &c. may be conveniently used for the treatment of paraffin sections, the slide with the section upon it being immersed in the fluid.

STAINING SECTIONS.

Where Gram's method is applicable it gives by far the best results, and should always be employed. When, however, the organisms are decolourised in Gram's process some other method must be adopted. One of the best is to stain for from ten minutes to six or eight hours in Löffler's methylene blue. Fresh easily staining organisms will be sufficiently stained in ten or fifteen minutes, but when the organism is difficult to stain, as glanders, six to eight hours may not be any too long a time. After staining, the sections are well rinsed in distilled water and then slightly decolourised by rinsing for half a minute or so in a watch-glass of distilled water to which a drop or two of glacial acetic acid has been added. They are then again washed and passed as rapidly as possible through alcohol, cleared in xylol, and mounted. Carbol-methylene blue may be used instead of the Löffler's solution, the staining taking from a few minutes to half an hour. If a contrast stain be desired, the sections may be treated for a few seconds with the eosin solution after the dilute acetic, but a better method is to use the Chenzinsky's solution. The sections, after being in absolute alcohol for a few minutes, are transferred to this solution for four or five hours. They are then well washed in distilled water until hardly any blue can be seen, and dehydrated, cleared, and mounted.

The micro-organisms in sections stained with Löffler's blue are very liable to become decolourised unless the dehydration is expeditiously performed. To avoid this, Unna's method may be adopted. After staining and decolourising with acidulated water as described, the sections are placed on the slide, gently warmed, and so dried; they are then treated with xylol and mounted in balsam. The tissue elements, however, are apt to suffer.

CAPSULE STAINING.

Many organisms, especially in the tissues, are invested with a capsule of gelatinous matter, probably derived from the membrane of the bacterial cell, and differing in composition in different species. The capsule may be as thick as the bacterial cell itself, and appears, in the unstained state or after staining by the ordinary methods, as a clear halo or zone surrounding the organisms. In order to stain the capsule, one of the following methods may be adopted:

1. Prepare cover-glass specimens in the ordinary way. Stain by just dipping in the following solution:

Carbol-fuchsin	1 part
Distilled water	1 part

Rinse in water and then stain for fifteen seconds in a very weak aqueous solution of gentian violet (0.1 per cent.). Rinse in water, dry, and mount.

2. *Ribbert's Method*.—Mix,

Water	100 parts
Alcohol	50 parts
Acetic acid	12.5 parts

Warm, saturate with dahlia, and filter. Stain in this for a few minutes, wash, dry, and mount. The bacteria are stained a deep, and their capsules a pale, blue.

3. *Friedländer's Method* (for tissues).—Mix,

Concentrated alcoholic solution of	
gentian violet	50 parts
Distilled water	100 parts
Acetic acid	10 parts

Stain the sections in this solution in the warm for twenty-four hours. Rinse well in 1 per cent. acetic acid, pass through alcohol and xylol, and mount in balsam.

SPORE STAINING.

When spore-bearing bacteria are stained by the ordinary methods, the spores remain uncoloured and appear as bright refractile bodies. This seems to be due to the fact that the spores are enclosed by a very resisting membrane which prevents the entrance of the staining agent. By staining by some method which causes the penetration of the stain and then cautiously decolourising, it is possible to decolourise everything except the spores, the resisting membrane of which in the same way prevents the full action of the decolourising agent.

(a) *Simple Method*.—A cover-glass specimen is prepared in the ordinary way. It is floated on a watch-glass of carbol-fuchsin, which is kept steaming by warming over a sand bath or small flame for twenty minutes. After being washed in water, it is rinsed for a second or two in 5 per cent. sulphuric acid and again washed at once in water. If there is still a good deal of the red colour remaining it may be given another rinse in the acid, but if nearly colourless it should be mounted in water and examined. If the spores alone are well stained the preparation may be removed, blotted, and then counter-stained with Löffler's methylene blue for one to two minutes, washed, dried, and mounted. If, however, the bacilli as well as the spores retain the red colour, the preparation must be further decolourised in the acid, while if everything has been decolourised, it may be re-stained in the warm carbol-fuchsin.

The spores sometimes stain better if the preparation be fixed by passing through the flame *twelve* times instead of three, as is usual.

Spore-staining often requires a good deal of patience, and in many instances it is very difficult to obtain a satisfactory preparation by this simple method, in which case that of Moeller should be made use of and rarely fails.

(b) *Moeller's Method*.—Prepare the cover-glass specimen in the ordinary way. Treat with absolute alcohol for two minutes, and then with chloroform for two minutes. Wash in water and treat with a 5 per cent. solution of chromic acid for two minutes. Wash, and then stain with warm carbol-fuchsin for ten minutes. Wash, and decolourise carefully in 5 per cent. sulphuric acid. Wash, and counter-stain with Löffler's methylene blue for one minute, wash, dry, and mount. Some organisms, such as the 'potato bacillus,' stain better if treated with the chromic acid for five to ten minutes.

FLAGELLA STAINING.

Many organisms possess delicate protoplasmic processes, flagella, in greater or less number; but they are not visible when the organism is examined in the living condition, nor when the ordinary staining methods are employed. In order to demonstrate them it is necessary to make use of some special method, in which a mordant is essential. One of the earliest devised was that of Löffler, which with care gave fair results. It is not, however, nearly so satisfactory as that known as Van Ermengem's, so will not be given here.

For all methods of flagella staining the cover-glasses must be absolutely clean, the cultures recent, and the growth sufficiently diluted to obtain the bacilli in an isolated condition.

(a) *Van Ermengem's Method*.¹—In order to clean the cover-glasses they should be boiled in the following solution :

Potassium bichromate	60 grams
Concentrated sulphuric acid	50 c.c.
Water	1000 c.c.

Then wash well in water and keep in absolute alcohol.

For use they should be placed with a clean forceps upright under a bell-jar and allowed to dry, not wiped.

The culture should be quite recent. In the case of typhoid or *b. megaterium*, which are good ones to practise on, an eighteen to twenty hours agar culture should be used. A loopful of the growth is removed and well rubbed up in a watch-glass of distilled water. Cover-glass specimens are prepared with a loopful of this dilution, *air-dried*, and then fixed by passing three times through the flame, holding in the fingers to avoid overheating. They are now treated with the following solution for half an hour at room temperature, or five to ten minutes at 60° C.:

- a Osmic acid (2 per cent. solution) . . . 1 part
- Tannin (10-25 per cent. solution) . . . 2 parts

Add to every 100 c.c. of the mixture four to five drops of glacial acetic acid.

Then wash well in distilled water and afterwards in alcohol.

Now immerse in the following solution for five to ten seconds:

- β Nitrate of silver (0.25-0.5 per cent. solution).

Then, without washing, treat with the following solution for a second or two:

- γ Gallic acid 5 grams
- Tannin 3 grams
- Fused potassium acetate . . . 10 grams
- Distilled water 350 c.c.

Return again to the silver bath (solution β), using a fresh amount, and move the specimens about gently until the solution commences to darken. Then wash thoroughly in distilled water, dry, and mount.

(b) *Pitfield's Method*.—This is a simple method and gives fair results, the flagella being quite visible though somewhat faintly stained.

Two solutions are prepared :

- A. Saturated aqueous solution of
alum 10 c.c.
- Saturated alcoholic solution of
gentian violet 1 c.c.
- B. Tannic acid 1 gram
- Distilled water 10 c.c.

The solutions should be made with cold water, filtered, and preserved in separate bottles. For use equal quantities are mixed together. The cover-glass specimens, prepared in all respects as in the Van Ermengem method, are flooded with the mixture and held over the flame until it nearly boils ; they are now laid aside, with the hot stain on them, for one minute, and are then washed in water. After washing, the preparations are flooded with anilin gentian violet for a second, washed in water, dried, and mounted.

Löffler's method will be found described in most of the text-books. Other methods are : McCrorie, *Brit. Med. Journ.* 1897, i. p. 971 ; Brown, *Journ. Roy. Micro. Soc.*, 1893, p. 268 ; Luksch, *ib.* p. 121.

PRESERVATION OF CULTURES.

Gelatin and agar culture may be satisfactorily preserved by the following method. A few drops of formalin are poured carefully into the tube, so as not to disturb the growth, and allowed to act for a few minutes. The excess is then poured off, the lower part of the wool plug is moistened with corrosive sublimate solution (1-1000) and returned to the tube, and some melted paraffin poured on to the top of the plug so as to seal the tube, and a rubber-cap applied to protect it. The cultures will retain their characters for many months after this treatment, and the growths of chromogenic organisms are not much altered by it. If the growth in the tube is liable to wash off with the formalin the lower part of the plug may be soaked in it, and the growth merely exposed to the vapour. Plate cultivations may also be exposed to the vapour and the lid of the dish afterwards cemented on.

PRESERVATION OF PATHOLOGICAL SPECIMENS.

These may be preserved in the ordinary way in spirit, but a much better method, by which the natural colour of the

specimen is retained, is the following. The specimens are first washed in water, and then placed in the following solution for twenty-four to forty-eight hours

Formalin	6 parts
Sodium chloride	1 part
Sodium sulphate	2 parts
Magnesium sulphate	2 parts
Tap-water	100 parts

After being taken from the formalin solution the specimens are placed in methylated spirit for ten minutes, and then in a fresh lot of methylated; in this the colour to a large extent returns, and they should be carefully watched and not allowed to remain in it for more than an hour. They are then transferred to a mixture of equal parts of glycerin and distilled water to which a dash of formalin has been added, and in this they are preserved (Jorres method).

The writer has preserved meat infected with prodigious very satisfactorily by the following method. Slices were cut off and placed in the formalin solution given above for a few hours. They were then well drained and placed in suitable glass capsules. Ordinary nutrient gelatin was melted and sufficient poured in to cover the specimens, and when it had set a little formalin was poured on and allowed to remain for a few days. It was then poured off and the glass top cemented down.

For further information on staining methods, &c., see *The Microtometist's Vade-Mecum*, Bolles-Lee; *Practical Histology*, Schäfer, and *Methods and Formulæ for Staining*, Squire. Also *Pharmaceutical Journal*, 1897, June 5, p. 491 *et seq.*

CHAPTER IV.

METHODS OF INVESTIGATING MICROBIAL DISEASES—THE IN-
OCULATION AND DISSECTION OF ANIMALS—HANGING-DROP
CULTIVATION—INTERLAMELLAR FILMS—THE MICROSCOPE.

THE systematic study of conditions due to the activity of micro-organisms is in many instances no light matter. When only one or two forms are present and these are readily cultivated it may be comparatively easy, but when there are many species the matter may become exceedingly complicated. The first step to take is to ascertain what organisms are present in the material by careful microscopical examination, both in the fresh condition and in stained preparations, and at different stages. In disease conditions, for example, the blood and secretions may be examined before and after death, but in the latter it must be remembered that soon after the fatal event adventitious organisms rapidly make their appearance, gaining access from the air and from the intestinal tract. If organisms be detected an attempt should be made to determine if there is any predominant form and if it be constantly present at different stages. If organisms be observed, so much the better; but if not, it cannot therefore be said that they are absent, for they may be few in number, and consequently be missed in a microscopical examination; or they may be confined to a particular locality or tissue, or are only to be met with at one stage of the affection. In addition to the microscopical examination cultures must be made on various media, those media being chosen which will probably be suited to the growth of the organism present

in the particular condition; for example, in examining the diseases of animals, media rich in proteid, such as blood serum, peptone-agar and gelatin, will be the most suitable. In examining the bacterial diseases of plants, infusions prepared from the plant itself, and enriched by the addition of vegetable proteids, and carbohydrates, should be chosen. In fermentation, beer-wort, grape or fruit juice, and saccharine solutions should be made use of; while for the nitrifying organisms, solutions containing salts, salts of ammonia, urea, and asparagin will have to be employed. In addition it will in most cases be necessary, and in all safer, to make plate cultivations, to isolate the various species, and to obtain pure cultures. Having obtained pure cultivations it will be necessary to determine the species of organism, if it has been previously described and isolated, or to give a careful description of it, if it be a new one, for the use of subsequent investigators. In the identification or description of an organism all the following points must be carefully noted:

1. The morphology of the organism under various conditions, its size, form, and motility, the presence of flagella, and their number and character.

2. The presence or absence of spore formation, its nature, and the conditions under which it occurs.

3. The peculiarities of staining, and its staining reaction with Gram's method.

4. The characters of the colonies in gelatin and agar or other media.

5. The characters of the growth on a variety of culture media at different temperatures—for example, for a pathogenic organism on blood serum, agar, and gelatin (surface and stab cultures), in broth and on potato; liquefaction or not of the gelatin; its growth in milk, with or without curdling.¹

¹ All organisms which liquefy gelatin curdle milk. Organisms which do not liquefy may curdle milk by the formation of acid, or in some cases perhaps by a special ferment.

6. Its behaviour towards oxygen—is it aërobic or anaërobic?

7. Its range of growth at different temperatures.

8. The production of acidity (by growing on neutral litmus sugar agar).

9. Its reducing power by growing in litmus broth which becomes decolourised, or by the formation of nitrites in a solution containing nitrates.

10. The production of gas (by a stab culture in sugar agar).

11. The production of indol with or without nitrites.

12. The production of pigment and the conditions under which it occurs.

13. Its pathogenic action on various animals if it be a disease germ, or the changes which it produces if it be an organism connected with other conditions.

14. Its chemistry and the chemical changes which it induces.

15. Its thermal death point and the action of germicides and antiseptics upon it.

It must never be forgotten that under cultivation the properties of organisms may be considerably modified and due allowance must be made for this. For example, pathogenic organisms may lose their virulence more or less completely, pigment production be lost, and fermentive action modified; an instance of the latter is given by Percy Frankland. A bacillus isolated by him possessed the power of fermenting calcium glycerate, but after cultivation on ordinary gelatin it completely failed to do so.

To obviate these difficulties the organisms should be cultivated under as nearly natural conditions as possible and sub-cultivation avoided as far as can be. No general rule can be given as to the duration of life of cultures on artificial media. Most organisms will retain their vitality for at least three or four weeks without being transferred to a fresh soil,

some for many months ; a few must be subcultured every week or they will die out ; while there are still a small number, as leprosy, and relapsing fever, which so far have never been cultivated.

For an organism to retain its virulence it is generally necessary to pass it through a susceptible animal at longer or shorter intervals, and to enhance the virulence recourse must be had to a succession of passages through susceptible and then less susceptible animals. In this way the virulence of organisms has been increased to a point far greater than is ever met with naturally, as in the case of the streptococcus pyogenes. If an organism retains its virulence, even slightly, it is generally possible, by employing large doses, to enhance this by a passage through a susceptible animal. Another method can also be adopted, namely, to inject along with it some other pathogenic form, such as the streptococcus pyogenes ; the combination will kill the animal, and the slightly virulent organism can be recovered and will be found to be increased in virulence. A third method is to inject the organism into a susceptible animal together with a lethal dose of toxin obtained from a virulent form of the same species. The slightly virulent organism will then be able to grow under the most favourable conditions, and a completely non-virulent form can be made to regain its lost virulence.

For the inoculation of animals various methods may be adopted. In some cases, after clipping the hair, the organism may be introduced by rubbing into the skin after scarification, or, a small incision being made through the skin, a small quantity of a culture may be introduced on a platinum needle ; or a broth culture or an emulsion, made with sterilised water or broth, may be injected with a sterilised syringe subcutaneously, intraperitoneally, or into the muscular or other tissues or organs as required, for the seat of inoculation has to be varied for the different species to produce their pathogenic effect. For injection purposes the Koch syringe

(fig. 18) is as good a form as any; two sizes are useful, 1 c.c. and 2 c.c. Another good syringe, permitting of thorough sterilisation, is one of the ordinary hypodermic form, in which the piston is made of a pad of asbestos. Before use the syringe with the needle should be boiled for ten minutes to sterilise it; after use it may be well rinsed with 1-20 carbolic solution and again boiled.

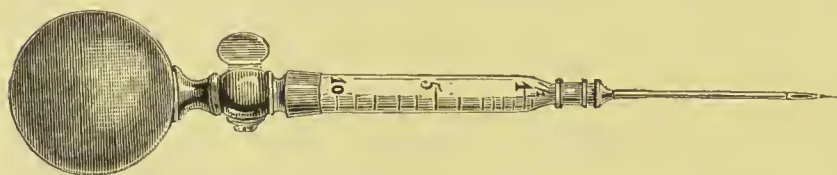


FIG. 18.—Koch SYRINGE.

Guinea-pigs and rabbits are usually inoculated in the thigh or abdomen; mice in the dorsal region or at the root of the tail (dorsally), the hair being clipped, and the skin disinfected if necessary with 1-20 carbolic solution. Numerous mechanical holders have been devised for the animals, but they are not usually required. Rabbits may be inoculated intravenously through the large dorsal vein in the ear. The hair is clipped, and the skin is well washed with 1-20 carbolic solution; the base of the ear is lightly pinched so as to obstruct the venous but not the arterial circulation, and render the vein prominent. A small incision is then made through the skin so as to expose the vein, and the injection made with a hypodermic syringe fitted with a fine needle, the needle being passed into the vein towards the base of the ear. After the withdrawal of the needle the wound is dressed with some antiseptic wool and collodion.

The examination of the dead animal should be carried out with as little delay as possible. For dissection, the body should be pinned out on the back on a board, by pins or nails through the feet, and the abdomen well soaked with 1-20 carbolic acid solution, not so much to sterilise the skin as to

prevent the hair from floating about and getting into the incision; to obtain thorough sterilisation of the skin it is preferable to clip or shave the hair and then sear with a red-hot iron. Two or three knives and pairs of forceps and scissors should be well rinsed with the carbolic solution and then placed in a beaker of water kept boiling during the dissection. An incision is made and the skin well reflected and pinned out; the knife and forceps should then be laid aside, and fresh sterile instruments taken for the deeper incision and opening the body cavities; these again are laid aside and a third set of instruments employed for incising the organs.

During the progress of the dissection the condition of the tissues at the seat of the inoculation should be noted, and likewise the conditions of the serous membranes and the various organs. In many diseases the organism is met with most abundantly in the spleen, in others in the blood, and in some at the seat of inoculation. Where a systematic examination is made, cover-glass specimens and cultures on two or three media, aërobic and anaërobic, should be prepared from the seat of inoculation, the spleen, liver, lungs, and heart-blood, and in some cases from the serous membranes, muscles, or central nervous system in addition, the carcase being in the intervals covered with a bell-jar which has been rinsed in, or with filter-paper moistened with, carbolic solution. An assistant is often useful or even necessary. The greatest care must be taken to avoid dropping or splashing or otherwise disseminating infective material, any stains being immediately swabbed up with carbolic solution; and the operator must exercise every precaution to prevent the infection of himself or others. The access of flies to the carcase must also be guarded against, as they might carry infection. When done with, the carcase should be efficiently disinfected and disposed of without delay, preferably by burning it, together with the board on which it has been pinned out.

When it is required to obtain the blood of an animal several expedients may be adopted. If the animal is not required to be kept alive for further experiment, it may be decapitated and the blood collected in a porcelain dish; but if a sample only is required, and the animal has to be further treated, as in antitoxin work, it may be bled from the carotid, the vessel being afterwards ligatured. In the rabbit a small artery passes superficially across the inner aspect of the thigh; this permits a small quantity of blood to be withdrawn without the necessity of an operation such as is required to expose the carotid.

Blood may be obtained from a patient for the Widal reaction, or for microscopical examination, or for culture experiments, by pricking the finger or the lobe of the ear with a sterile needle or lancet, or with half a steel pen (nib); the skin having been first disinfected by washing with soap and water, then with 1–20 carbolic solution, and finally, to remove the antiseptic, with absolute alcohol followed by ether.

Although the modern methods of isolation and cultivation have rendered immense service to bacteriology, they have also had the effect of diminishing the attention paid to the exact morphology and biology of organisms. At the present time there is a tendency to investigate bacteria *en masse* rather than to study them as individual living forms, and the following remarks by Marshall Ward¹ may be aptly quoted in this connection:

‘ We must remember that De Bary and Brefeld had aimed at obtaining a single spore isolated under the microscope, and tracing its behaviour from germination continuously to the production of spores again; and when we learn how serious were the errors into which the earlier investigators of the mould fungi and yeasts fell, owing to their failure to trace the development continuously, from spore to spore, and the triumphs obtained afterwards by the methods of pure

¹ Address in Botany, *Brit. Assoc.* 1897. *Nature* 56, 1897, p. 455 *et seq.*

cultures, it is not difficult to see how inconclusive and dangerous all inferences as to the morphology of such minute organisms as bacteria must be unless the plant has been so observed. As a matter of fact, the introduction and gradual specialisation of Koch's methods of rapid isolation of colonies encouraged the very dangers they were primarily intended to avoid. It was soon discovered that pure cultures could be obtained so readily that the characteristic differences of the colonies in the mass could presumably be made use of for diagnostic purposes, and a school of bacteriologists arose who no longer thought it necessary to patiently follow the behaviour of the single spore or bacillus under the microscope, but regarded it as sufficient to describe the form, colour markings, and physiological changes of the bacterial colonies themselves on and in different media, and were content to remove specimens occasionally, dry, and stain them, and describe their forms and sizes as they appeared under these conditions. To the botanist, and from the points of view of scientific morphology, this mode of procedure may be compared to what would happen if we were to frame our notions of species of oak or beech according to their behaviour in pure forests, or of a grass or clover according to the appearance of the fields and prairies composed more or less entirely of it, or—and this is a more apt comparison, because we can obtain colonies as pure as those of the bacteriologist—of a mould fungus according to the shape, size, and colour, &c. of the patches which grow on bread, jam, gelatine, and so forth.'

EXAMINATION IN THE FRESH STATE.

One essential stage in the investigation of an organism is its examination in the fresh and living condition. This may be done by placing a droplet of sterile water, broth, or other fluid on the slide, inoculating with a trace of the organism or growth, and covering with a cover-glass and examining

microscopically. The action of stains and reagents on the organisms may be observed by the irrigation method. A drop of the stain or reagent (C, fig. 19) is placed on the slide A, just in contact with one margin of the cover-glass, B, and is drawn through the preparation by means of a small piece of filter-paper, D, placed on the other side, a torn margin touching the film of fluid at one edge of the cover-glass.

The filter-paper absorbs the fluid from under the cover-glass, leaving the cells and other particles behind, and at the same time the reagent on the opposite side flows under the cover-glass to take the place of the absorbed fluid. Afterwards the excess of the reagent or stain may be washed away by running in water in a like manner under the cover-

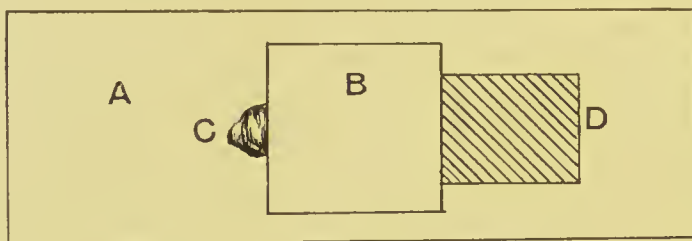


FIG. 19.—METHOD OF IRRIGATION.

glass. Care must be taken not to get any fluid on the upper surface of the cover-glass, which must always be kept dry. The advantage of this method is that it can be applied while the specimen is being examined under the microscope, and the action of the reagent on a particular cell or granule can, with a little care, be watched.

If the specimen has to be kept for any length of time the film of fluid will before long evaporate and become dry. To prevent this a ring of oil or vaseline may be painted round the margin of the cover-glass so as to seal it to the slide. A simple method of keeping organisms under examination for a lengthened period of time, and of watching their growth and development, is the hanging-drop cultivation.

A hollow-ground slide is sterilised by passing several times through the Bunsen flame. A fairly thick ring of vaseline is then painted round the margin of the hollow. A cover-glass is similarly sterilised by passing five or six times through the Bunsen, care being taken not to heat sufficiently to melt it. A droplet of some sterile fluid medium—water, broth, wort, sugar solution, &c.—is then placed in the centre of the cover-glass with the looped platinum needle, which must of course be previously sterilised by heating. This droplet is then inoculated with the organism which is to be observed. The hollow-ground slide is now taken and turned over, so that the ring of vaseline is downwards, and then placed on the cover-glass, so that the droplet is

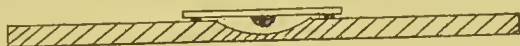


FIG. 20.—HANGING-DROP CULTIVATION.

situated in the middle of the hollow, but not touching the slide at any point. The cover-glass adheres to the slide by means of the vaseline, and on quickly inverting the whole, so that the fluid has no time to run, it will be found that the droplet is hanging from the under surface of the cover-glass in a cell which is hermetically sealed by the vaseline, and evaporation is thus rendered impossible (fig. 20). Such a preparation in fact can be kept for a week or ten days in a warm incubator without drying up. Great caution must be exercised in examining a hanging-drop cultivation microscopically, especially with the immersion lenses, for unless care be taken the cover-glass is apt to be cracked or broken.

The hanging-drop cultivation is the only certain method of ascertaining whether an organism is motile or not, the motility of an organism being often an important clue to its identification. Actively motile organisms may frequently be met with in a resting stage, although still alive, and various factors may bring about this condition, such as old age,

exhaustion of nutriment, excessive heat or cold, electric shocks, and the like. The absence of movement in an organism in an ordinary microscopical specimen does not necessarily prove that it is non-motile. A hanging-drop cultivation should be prepared and placed under conditions of temperature, &c. favourable to the growth of the organism, and examined after an interval of an hour or so, or better still at intervals of half an hour for three or four hours. In this time the old cells will revivify, and new ones will have been produced, and if the organism be a motile one, more or less active movement of some of the cells is almost sure to be observed. It is necessary to beware of two fallacies in connection with motility—not to mistake for it the so-called Brownian movement, which is a vibratory one backwards and forwards about one point, and common to all fine particles suspended in a fluid; and not to be misled by a flotation of the cells due to currents set up in the fluid from some cause or other—all the particles then moving *in the same direction*.

Another important purpose for which the hanging-drop cultivation may be employed is that of obtaining a permanent record of the various phases through which an organism may pass during its development. If a number of these cultivations be made, say twenty, in an exactly similar manner, and afterwards kept under identical conditions, and if at the end of every half-hour one of the preparations be taken, its cover-glass carefully removed, and the droplet dried and stained, a permanent record of the life-history of the organism is obtained extending over ten hours.

Dunham¹ has devised an ingenious method of anaërobic hanging-drop cultivation. A piece of narrow glass tubing is bent into a U; one end of the U just at the bend is then drawn out in the blowpipe flame, forming a capillary portion, and the other end is bent over so that the whole tube is now crank-shaped. It is sterilised in the flame and the tip

¹ *Johns Hopkins Hosp. Bull.* 1897.

of the capillary portion broken off with a sterile forceps, and a little of a broth culture of the organism to be examined is drawn up into it, and the tip again sealed in the flame. Into the other wide and open end of the tube a little solid pyrogallic acid is packed and moistened with a 20 per cent. solution of caustic potash, and the tube closed by slipping on a small piece of rubber tubing and stoppering with a short length of glass rod. The capillary portion can then be examined under the microscope, the preliminary bending into a U being to flatten the tubing.

The observation of hanging-drop cultivations at blood-heat can be carried out on some form of warm stage.

*Interlamellar Films.*¹—Another method of investigating the life-history of organisms, especially moulds and protozoa, is by means of interlamellar films. A glass slide $1\frac{1}{2}$ inch by 3 inches is made hot, and on its upper surface three small drops of sealing-wax are placed, so arranged that they form the apices of an equilateral triangle, the side of which measures about one inch. A cover-glass of about $1\frac{1}{4}$ inch in diameter is then taken and sterilised in the Bunsen flame, and a droplet of a suitable nutrient medium deposited upon it, which is next inoculated with the organism to be observed. The slide is also sterilised in the Bunsen flame, a droplet of the nutrient medium is placed in its centre between the points of sealing-wax, and the cover-glass picked up with sterilised forceps, inverted, and lowered on to the slide. The nutrient medium is thus contained between the slide and the cover-glass, and by using a hot wire, and so softening the sealing-wax, it can be spread out to form as thin a layer as desired. The preparation is kept in a moist chamber to prevent evaporation and can be studied when required. By cementing a strip of glass to each end of the slides several of the interlamellar preparations can be stacked one upon another in the moist chamber.

¹ Delepine, *Lancet* 1891, i. June 13.

THE MICROSCOPE.

The most important of all the instruments employed in the investigation of micro-organisms, the microscope, must next be considered.

A bacteriological microscope should be of the monocular form, and have a rack and pinion coarse adjustment and an efficient fine adjustment. The stage should be large and roomy and quite plain, with two or more holes at its margin to receive spring clips for fixing the slide. *As a rule*, the mechanical stage should be avoided, and if one is purchased,

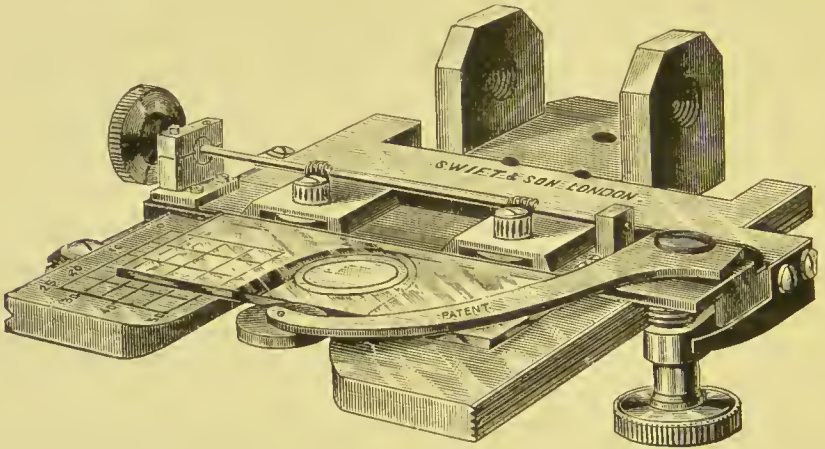


FIG. 21.—SWIFT'S DETACHABLE MECHANICAL STAGE.

some form which is *detachable* should be chosen. The mechanical stage hampers that freedom of manipulation which is so useful in rapidly looking through a specimen, while by practice the fingers become educated to perform the finest movements. Undoubtedly in many cases a mechanical stage is very useful, but the detachable form is to be preferred (fig. 21), so that, if required, the stage may be disencumbered for the examination of the plates used for plate cultivations, &c. The best material for the stage is vulcanite, which is preferable to either glass or brass.

A sub-stage condenser is essential in all work in which high powers are employed, and also enhances the value of low powers. It consists of a lens or system of lenses below the stage, by means of which the light is concentrated on the object. It should have a rack and pinion, or screw adjustment, for focussing, and be provided with some form of diaphragm for modifying the light, by far the best being the 'Iris,' which is well worth a slight extra cost. To obtain the best results the condenser must be centred—that is, adjusted so that its optical axis corresponds to the optical axis of the objective; and for this purpose it is usually provided with two lateral screws working at right angles to each other, by means of which its position relative to the optical axis can be altered. Some makers rule two fine cross-lines on the upper surface of the lens of the condenser, in which case all that is necessary in order to centre is to focus these lines with a low power—say a 1-inch objective—and then, by turning the lateral screws, the point of intersection of the cross-lines is brought into the centre of the field. If, however, the condenser has no cross-lines, a very small diaphragm should be used, and the hole focussed with a low power; then similarly, by working the lateral screws, the hole of the diaphragm is brought into the centre of the field. Below the sub-stage condenser a mirror with concave and plane surfaces should be fitted, the *plane* surface being used with the condenser, as a general rule. The necessity for careful illumination must be insisted upon; in fact, to obtain the best results it is necessary to arrange the light for every specimen, altering the inclination of the mirror and the aperture of the diaphragm and focussing the condenser. A good specimen may be utterly spoilt, visually, by bad illumination; while an indifferent one may be made to look respectable by careful illumination. In examining micro-organisms, such as yeasts or bacteria, in the fresh or living and unstained condition, it is necessary

as a rule, to diminish the light by means of a small diaphragm; while for stained or opaque objects the full aperture of the diaphragm, or thereabouts, may generally be employed. The microscopist should accustom himself to examine specimens by both daylight and artificial light; as regards the latter, probably no form surpasses a paraffin lamp with flat wick, the *edge* of the flame being always used. For the finest work, daylight illumination is inadmissible.

Two eye-pieces are quite sufficient, and it is better to have the lower-power ones, such as the B and C of the English, or the 2 and 3 of the Continental makers. Although increased magnification can be obtained by the use of a high-power eye-piece, it is at the expense of definition, the image losing its sharpness, because the eye-piece magnifies the image formed by the objective, and any imperfections in the latter are made more apparent, so that the use of very high eye-pieces is not to be recommended, except with the finest lenses, and, moreover, as will be pointed out later, it is useless to increase the amplification beyond a certain point.

With regard to the length of the tube of the microscope, this differs in the English and Continental systems. The standard English tube-length is 8.75 in., the Continental 6.3 in. The latter is much more convenient for working purposes, but the finest objectives are now usually adjusted for the English length. The tube of the microscope is generally provided with an inner, or draw-tube, by means of which its length can be nearly doubled; this gives increased amplification, but at the expense of definition, at least with the higher powers which are corrected or adjusted for a definite tube length.

The lenses or objectives must next be considered.

For powers higher than the $\frac{1}{8}$ in., or thereabouts, it is advisable, for many reasons, to employ the immersion system of objectives. With these lenses a drop of either water, in the

water immersion system, or of cedar oil, in the oil immersion one, is placed on the cover-glass, and the objective is racked down so that its front lens touches and is immersed in either the water or oil, as the case may be. It is a good plan to then raise the objective very slightly by means of the coarse adjustment, still, however, keeping it in contact with the drop of water or oil. The observer then, looking down the microscope, very cautiously and gradually racks down again with the coarse adjustment until the object comes into view, and finishes the focussing with the fine adjustment. The fine adjustment should only be used after the object has been brought into view by means of the coarse adjustment. After the examination has been concluded for the day, the lens should be carefully wiped with a soft rag to remove the water or oil. If the oil should happen to dry on the lens, it may be removed by wiping with a rag moistened with alcohol, and quickly drying with another rag. The immersion system of objectives has many advantages—the loss of light is less, the distance between the cover-glass and the front of the objective—the working distance, as it is termed—is greater, and more can be seen with an immersion lens than with a dry lens of equal magnifying power. This can best be illustrated by means of two simple diagrams.

In fig. 22 let cd represent the surface of a fluid, either water or oil, and let ab be drawn perpendicular to this surface, and cutting it at y . Let ry represent a ray of light proceeding from a rarer medium, such as air, into a denser one, water, or oil. As is well known, this ray when it enters either the water or the oil does not continue in the same direction, but is 'refracted' or bent nearer the perpendicular ab , the bending being more marked with oil than with water. Thus we may suppose that the direction of the ray in water would be represented by the line yw , and in oil by the dotted line yo . Conversely, a ray of light proceeding from a denser medium into a rarer is bent away

from the perpendicular, and the rays wy in water, and oy in oil, would, on emerging into air, proceed in the direction yr .

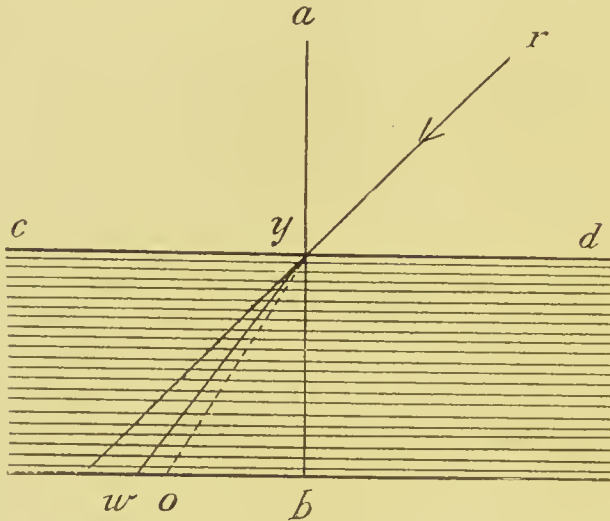


FIG. 22.—DIAGRAM TO ILLUSTRATE THE REFRACTION OF LIGHT.

In fig. 23 (which, for convenience, is drawn somewhat out of proportion) let s represent an ordinary glass micro-slide, x a layer of canada-balsam in which the object is mounted, and covered with the cover-glass G , while L is the objective with its front lens. Let the object be illuminated by the ray of light Yy ; this on entering the glass of the slide and the canada-balsam will be refracted or bent nearer the perpendicular and will proceed in the direction yt . Canada-balsam, and also cedar oil, produce about the same amount of 'refraction,' or bending in a ray of light, as crown-glass, and hence these three substances—crown-glass, canada-balsam, and cedar oil—are said to have the same 'refractive index,' and, consequently, the glass of the slide, the canada-balsam and the cover-glass, act as one homogeneous medium, and the line yt is a straight one. In the first place, let us suppose that the objective L is a dry one, having a layer of

air between its front lens and the cover-glass; then the ray of light, on emerging from the cover-glass into the air, is again bent away from the perpendicular and pursues a direction practically parallel to its former one, represented by the line *t w*, and misses the lens altogether—the lens is unable to take it up. If, however, we suppose that our objective is an oil-immersion one, and that a drop of cedar oil takes the place of the layer of air between the cover-glass and the front lens in the foregoing example, then the glass slide, canada-

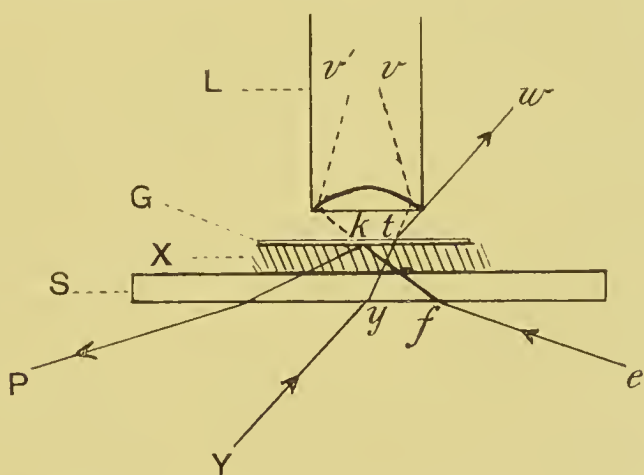


FIG. 23.—DIAGRAM TO ILLUSTRATE THE COURSE OF RAYS OF LIGHT
THROUGH AN OBJECTIVE.

balsam, cover-glass, cedar oil, and front lens of the objective, form practically one medium; they all have the same refractive index and produce the same amount of refraction or bending in a ray of light. Therefore the direction of the ray forms a straight line in all these, and the ray passes into the objective as is represented by the broken line $t-v$. More important still, however, is what happens to rays which fall on the slide at a very oblique angle. In the same figure (fig. 23) let ef represent such a ray; on entering the slide it will be refracted, and its passage through the slide, balsam,

and cover-glass may be represented by $f k$. As before, let us suppose that in the first place our objective is a dry one, and that we have a layer of air between the cover-glass and its front lens. In this case, if the angle which $f k$ makes with the perpendicular is greater than about 39° or 40° the ray, instead of emerging from the cover-glass into the layer of air, is totally reflected by the cover-glass and pursues a course roughly represented by $k r$, so that it never gets near the objective. If, however, we employ an oil-immersion objective, with oil instead of air between the cover-glass and its front lens, then, as before, the slide, balsam, cover-glass, oil, and front lens of the objective, form practically one homogeneous whole, and the ray $e f k$ instead of being totally reflected continues its course in a straight line, and is taken up by the objective, as is represented by the dotted line $k-v'$. Hence we see that the same rays which are unable to enter a dry objective are taken up by an oil-immersion one, and that an oil-immersion lens can take up rays which fall on the slide at a very oblique angle.

In order that these oblique rays may be present, ready to be taken up by the oil-immersion objective, it is necessary to employ a sub-stage condenser. It is only by means of a sub-stage condenser that a 'wide-angled cone of rays,' as it is termed, is obtained. Hence to make full use of an oil-immersion objective—to 'get the most out of it'—it is absolutely essential to employ a sub-stage condenser. It will also be obvious that although a water-immersion objective admits more rays than a dry one, it does not admit so many as an oil-immersion. It must be pointed out, however, that canada-balsam, or some medium having about the same refractive index, must be used for mounting to obtain the full advantage of the oil-immersion system. The most serviceable oil-immersion lens for general use is the $\frac{1}{12}$ inch. The oil-immersion can of course be used for examining objects mounted in water, &c. cedar-oil being

still used between the cover-glass and the lens. It is to be noted that a dry objective cannot be used as an immersion one, nor an immersion objective dry, the construction being different in the two cases.

The lenses in the objective are formed by cementing together different kinds of glass in order to correct for 'spherical' and 'chromatic' aberration. The rays passing through the margin and the centre of a simple lens are not focussed at the same point, and a distorted image is the result; this is known as 'spherical aberration,' while the violet and red ends of the spectrum being of different refrangibility, and a simple lens acting like a prism, coloured fringes are observed; this is termed 'chromatic aberration.' Recently, apochromatic objectives have been introduced, in which these defects are very perfectly corrected by the use of special glass and of fluorite. They are, however, expensive, often not durable, and are not necessary for ordinary bacteriological work.

In consequence of certain optical principles, the 'diffraction' theory, for details of which the reader must refer elsewhere,¹ it is useless to increase the magnifying power of a dry objective beyond a certain point; for, although the object viewed appears *larger*, no more *details of structure* can be made out.

The use of the immersion system increases the 'resolving power,' or the amount of detail which can be seen. Thus, if a number of fine parallel lines be ruled on a glass plate, it is impossible to see with a dry lens more than about 96,000 lines to the inch as isolated lines. If more are ruled they will not appear singly, but will run together, and practically nothing is visible. With a water-immersion objective it is possible to see about 128,000 lines to the inch, and with an oil-immersion as many as 146,000 lines to the inch as separate lines—a clear gain in resolving power in the latter case of about one-half over a dry lens. As it is necessary, in order

¹ See *Carpenter on the Microscope*, edited by Dallinger.

to see such fine structures as lines ruled 50,000 or more to the inch must be, to have considerable amplification in addition to resolving power, not much is gained, in ordinary work, at any rate, by adopting the immersion system for the lower power objectives, such as the $\frac{1}{6}$ in.

There is no real necessity for the immersion objective, for bacteriological work, to be provided with a 'correction collar.' The 'correction collar' is an additional screw in the objective by means of which the distance between some of its constituent lenses can be altered to 'correct' for varying thicknesses of cover-glass, &c., and though necessary with the higher power dry lenses, it is theoretically unnecessary with the immersion system. Nevertheless, as slight variations do occur in the various media, glass, oil, &c., and they may not form a truly homogeneous whole, for the finest work the correction collar is still desirable. So much for the high-power objectives. As regards the lower powers, which, of course, are dry, a $\frac{2}{3}$ in. and a $\frac{1}{6}$ in. are very necessary ones to have. The $\frac{2}{3}$ in. is a more serviceable lens than the 1 in., which is generally recommended. A very useful accessory is a 'double' or 'triple nose-piece.' This consists of a light metal framework, which is attached to the lower end of the tube of the microscope, on to which two or three objectives can be screwed. The framework can be rotated, thus bringing each objective in succession into the optical axis of the instrument, and does away with the necessity for unscrewing and screwing on each time an objective is changed. A microscope such as described, with sub-stage condenser, two eye-pieces, a $\frac{2}{3}$ in. and a $\frac{1}{6}$ in. dry and a $\frac{1}{1\frac{1}{2}}$ in. oil-immersion objectives, triple nose-piece, &c., complete in case, cannot be obtained for less than about 15*l.*, and it is well to add an extra pound or two for superior finish. It may seem to be a good deal of money to expend, but it pays in the long run, and the instrument should last a lifetime, and can be used not only for bacteriology, but for almost any microscopical work, and

many a pleasant and profitable hour may be passed in its company. Both English and Continental firms supply microscopes arranged as indicated, and in this department the English makers hold their own.

The measurement of micro-organisms is carried out by means of a stage or an eye-piece micrometer. The former consists of a scale of tenths and hundredths of a millimetre or hundredths and thousandths of an inch ruled in fine lines on a glass plate, by means of which the measurements can be made by focussing the scale under the microscope. A useful adjunct is a disc of glass ruled with equidistant fine lines, which can be placed in the eye-piece, and the value of these divisions is ascertained by means of the stage micrometer. The stage micrometer is then removed and the object to be measured put in its place, and its dimensions determined by means of the eye-piece scale. The unit for microscopical measurement is the micron (sometimes erroneously termed a micro-millimetre), which measures one-thousandth of a millimetre, or $\frac{1}{25000}$ of an inch, nearly, and is designated by the sign μ .

CHAPTER V.

IMMUNITY—ANTITOXINS AND ANTITOXIN TREATMENT.

No fact in biology is more striking than the differences in susceptibility to disease conditions exhibited by different races and different animals. For example, the native races in many parts of the world are comparatively insusceptible to yellow and enteric fevers and malaria, the dog and goat are rarely affected with tuberculosis, and tetanus is never met with in the fowl; and to come nearer home, while some individuals are lucky enough to escape most of the commoner infectious fevers, others seem to contract them on every possible occasion, and to suffer from all the ills that flesh is heir to. These instances show that there is often a natural insusceptibility to infective disease, or a natural immunity, as it is termed, either complete or partial and unvarying at all ages, 'racial immunity,' or frequently varying at different ages, 'individual immunity,' as in the case of diphtheria and scarlatina, which become more and more rare as age advances.

Still more striking, perhaps, is the fact that an insusceptibility may be acquired either after an attack of infective disease or in certain instances by inoculation. Thus second attacks of small-pox, enteric fever, and scarlatina are rare, while inoculated small-pox and vaccinia protect against an attack of variola. These are examples of an acquired insusceptibility or acquired immunity.

With regard to the immunity of native races to certain diseases, this is probably due to natural selection and heredity;

during long periods of time the individuals being all exposed to the same risks, the susceptible ones are weeded out, while the survivors transmit their insusceptibility to their descendants; but this of course does not explain the reason for the relatively greater immunity of the insusceptible individuals. Various factors doubtless conduce to natural immunity. Frogs, fish, and chickens are naturally immune to anthrax. In the one case the body temperature is low, 18 C°. or thereabouts; in the other it is high, 40° to 41° C., and this may influence the growth of the anthrax bacillus, preventing its full and rapid development, which may be necessary for the production of the disease. That such is the case would seem to be shown by experiments in which the temperature of the medium is raised or lowered, infection then taking place; frogs and fish kept in water raised to a temperature of 35 C°, and chicken refrigerated so as to reduce their temperature, all perish from anthrax after inoculation. That this, however, is not necessarily the only factor is certain, for sparrows, which have a temperature as high as that of chicken, can be infected with anthrax without refrigerating. In no case probably is immunity absolute; that is, that infection cannot be induced under any conditions. Thus fowls, which are highly refractory to tetanus and tolerate considerable doses of tetanus toxin with impunity, can be tetanised with large doses of an active toxin; white rats, which are insusceptible to anthrax, become susceptible after fatigue, or when fed on an exclusively vegetable diet. Behring would ascribe the immunity of white rats to anthrax to the high alkalinity of their blood, and claims to have shown experimentally that a vegetable diet reduces this, and fatigue is said to act similarly.

The blood, lymph, and other fluids and tissue juices undoubtedly exert a more or less germicidal action on bacteria experimentally *in vitro*, and probably also in the body. But in this respect there is often a marked difference between the circulating blood and the blood *in vitro*.

In 1872 Lewis and Cunningham showed that bacteria injected into the circulation rapidly disappear. Of twelve animals injected, six hours afterwards bacteria could only be found in seven; of thirty-six animals, after twenty-four hours in only fourteen; and of seventeen animals, after from two to seven days in only two.

In 1874 Traube and Gschiedlen found that arterial blood, taken with antiseptic precautions from a rabbit into whose jugular vein 1.5 c.c. of a fluid rich in putrefactive germs had been injected forty-eight hours previously, failed to undergo decomposition for months. They attributed the germicidal action to ozonised oxygen. Similar results were obtained by Fodor and Wysokowicz, who supposed, however, that the bacteria lodged in the capillaries. The first experiments with extra-vascular blood were made by Grohmann. He found that anthrax bacilli after being kept in plasma became less virulent.

In 1877 Fodor injected 1 c.c. of an anthrax culture into the jugular vein of a rabbit, and one minute afterwards made plate cultivations, with eight samples of blood obtaining only one colony. Then with a sterilised pipette he took blood from the heart and added anthrax bacilli to it. This was kept at a temperature of 38° C., and plates being made from time to time showed a rapid diminution of germs. Prudden found that ascitic and hydrocele fluids restrained the development of certain germs, and Rovighi observed that the germicidal action of the blood was increased in febrile conditions.

Behring and Nissen found that the serum of the white rat, dog, and rabbit destroyed the *bacillus anthracis*, but serum from the mouse, sheep, guinea-pig, chicken, pigeon, and frog had no action. Thus, while the rabbit is highly susceptible to anthrax, its serum is germicidal; the chicken, on the other hand, is immune to anthrax, but its serum is inactive. Hence there is a considerable difference between the action of circulating and of extra-vascular blood.

Halliburton prepared from the lymphatic glands a cell-globulin β . Hankin found that this had marked germicidal properties, and concluded that this substance was probably the germicidal constituent of the blood serum. Bitter, who repeated Hankin's experiments, failed, however, to confirm them.

In 1890, Buchner with Voit, Sittmann, and Orthenberger obtained the following results :

1. The germicidal action of blood is not due to phagocytes.
2. The germicidal action of cell-free serum must be due to the soluble constituents.
3. The germicidal action of blood is not affected by neutralising, by pepsin, or by oxygen.
4. The dialysis of serum against water destroys its activity, but with 75 per cent. of sodium chloride it does not. In the diffusate there is no germicidal constituent, hence the loss of activity with water must be due to the withdrawal of inorganic salts.
5. In serum diluted with water activity is destroyed, but in serum diluted with salt solution activity is unaltered.
6. Inorganic salts themselves have no germicidal action. Hence it is the proteid constituents which are active.

Nuttall in 1888 used defibrinated blood of several animals, rabbits, mice, pigeons, sheep, and found that it destroyed the *bacillus anthracis*, *bacillus subtilis*, *bacillus megaterium*, and *micrococcus pyogenes aureus*. He confirmed Fodor's results, which also showed that after a while the blood loses its germicidal properties and becomes a suitable culture medium. Nissen continued this work and arrived at the following conclusions :

1. The addition of small quantities of salt solution or bouillon does not destroy the germicidal properties.

2. Cholera germs and Eberth's bacilli are easily destroyed by fresh blood.

3. For a given volume of blood there is a maximum number of bacilli which can be destroyed.

4. Peptonised blood (non-coagulated) is still germicidal.

5. In blood mixed with a 25 per cent. solution of magnesium sulphate (non-coagulated), the germicidal properties are weakened.

6. Filtered horse blood plasma is germicidal.

Christmas prepared a germicidal substance from the spleen by the following method: An animal was killed with ether, opened with antiseptic precautions, and the organ removed, cut into fine pieces, and covered with 50 c.c. of glycerin and allowed to stand for twenty-four hours, and then filtered. The filtrate was precipitated with five times its volume of alcohol and the fluid immediately decanted. The precipitate was washed with absolute alcohol to remove the glycerin, the traces of alcohol were removed by pressure, and the preparation was dissolved in 25 c.c. of distilled water. Air was next driven through this solution for some hours to remove the last traces of alcohol, and the germicidal properties were then tested.

Bitter examined this method, and his results in the main confirmed Christmas.

This survey clearly shows that the germicidal action of the fluids of the body may be an important factor in the production of natural immunity, at any rate, towards the living germs. It is clear, however, that this theory cannot explain immunity to bacterial toxins unless we assume that a destructive action on toxins is co-existent with germicidal action.

Another important theory of immunity is the doctrine of Phagocytosis, so ably supported by Metschnikoff. It has as its basis the following fundamental facts:—Firstly, the leucocytes in the circulating blood ingest and destroy any

foreign particles injected ; secondly, any injury to the tissues is immediately followed by an inflammatory reaction, in which the leucocytes escape from the vessels by diapedesis and congregate at the injured spot. Similarly, in many instances the leucocytes rapidly congregate at the seat of a bacterial infection, and approach and engulf the bacteria in the same manner as they do other foreign particles, and so rid the body of the unwelcome guests.

The migration of the leucocytes towards the scene of action is explained by Metschnikoff on the hypothesis that the chemical substances elaborated by the bacteria attract the latter and exert what he termed 'positive chemotaxis.' In this case the bacteria are removed by the leucocytes and general infection and death do not occur. But unfortunately in other cases the bacterial chemical products repel the leucocytes and 'negative chemotaxis' occurs, so that the bacteria are free to grow and multiply, and general infection ensues. That positive and negative chemotaxis really exist has been abundantly proved, and the process by which the bacteria are engulfed by the leucocytes can be readily watched. The leucocytes which act in this manner are termed phagocytes, and they are of two classes—the macrophages, the large uninuclear leucocytes, and the smaller microphages with a multiple or divided nucleus. That an acquired immunity may be due to the education of the leucocytes seems to be shown by the fact that, while in ordinary susceptible rabbits infection with anthrax is followed by a feeble phagocytosis, and the animals succumb, in vaccinated rabbits phagocytosis is very active. Moreover, in an animal refractory to anthrax, such as the frog, if the bacilli be enclosed in minute bags of paper or membrane, so as to prevent the access of the phagocytes, the bacilli grow and multiply. An immense amount of experimental proof has been obtained by Metschnikoff and his pupils of the truth of the doctrine of phagocytosis ; but in spite of their ingenuity, there are many objections

to the theory, and certainly phagocytosis *alone* will not explain many cases of immunity, and especially that towards bacterial toxins, the bacteria themselves being absent.

Vaughan, Novy, and McClintock have shown that the nucleins are powerful bactericides. Throughout their papers they use the term 'nuclein' in its widest sense. The nucleins are a group of organic compounds of complex composition containing phosphorus, and obtainable from animal cells. They are classified by Halliburton¹ in the following manner:—(a) Nuclein which contains no proteid (this is termed nucleic acid, and is met with in the heads of the spermatozoa); (b) Nuclein rich, and (c) Nuclein poor in nucleic acid. The former occurs in the nucleus, the latter in the nucleolus. (d) Nucleins containing little nucleic acid and chiefly proteid in nature. These are the so-called nucleo-albumins, and are found in the nucleus and in cell bioplasm generally; also in milk, eggs, &c. They are likely to be mistaken for globulins or mucin; the 'mucin' of bile, for example, being in reality a nucleo-albumin.

Vaughan, Novy, and McClintock² prepared 'nucleins' from the testes and thyroid, &c., and from yeast; their method was to strip the glands of their membranes, and digest with pepsin and .2 per cent. hydrochloric acid for several days. The undigested portion was then washed and dissolved in a .5 per cent. caustic potash solution. Tubes containing some of this solution were inoculated with various micro-organisms, *staphylococcus pyogenes aureus*, *bacillus anthracis*, &c. and plate cultivations were then made from the tubes at short intervals. After the lapse of an hour, in almost every instance, not a single organism remained alive.

In another paper Vaughan³ discusses the nature of the

¹ 'The Chemical Physiology of the Animal Cell' (*Gulstonian Lectures*, 1893), p. 20.

² *Medical News* (New York), May 1893, p. 536.

³ *Ibid.* October 1893, pp. 393, 421.

immunising agent in infective diseases. He believes that some constituent of the bacterial cells themselves is the immunising agent, and that this substance is a nuclein. He points out that in the methods employed for producing artificial immunity, such as by the injection of filtered or of attenuated cultures, &c., the germ substance is introduced either in the cellular form or in solution. Klein¹ has shown that an injection of one of the following six organisms—(1) Koch's comma, (2) Finkler-Prior's comma, (3) *B. coli communis*, (4) *Proteus vulgaris*, (5) *B. prodigiosus*, (6) *B. typhosus*—will protect an animal against either of the remaining five. He therefore concludes that there is an immunising agent common to all these six organisms, and that this substance is intra-cellular and a constituent of the bacterial cells themselves. Klein's observations are interesting in connection with the foregoing experiments. With regard to immunity conferred by the blood serum of an immunised animal, Vaughan is also of opinion that a nuclein is present in the serum, and is the active agent.

Lastly, Vaughan and Novy² have also investigated the bactericidal constituent of the blood serum, and have come to the conclusion (*a*) that it is not the serum-albumin; (*b*) that it is a proteid, for it is destroyed at 65° C.; and (*c*) that it is probably a nuclein, for it is not destroyed by gastric digestion. Acting on this supposition, Vaughan and Novy by further experiments have succeeded in isolating a nuclein, which was found to possess powerful bactericidal properties when tested on Koch's comma, *S. pyogenes aureus*, and anthrax; they precipitated blood serum with a mixture of ether and alcohol, and digested the purified precipitate with pepsin and .2 per cent. hydrochloric acid with this favourable result. They suggest that the nuclein is derived from the disintegration and solution of leucocytes during the coagulation of the blood, and

¹ *Trans. Path. Soc. London*, 1893, p. 220.

² *Med. News*, December 1893, p. 701.

that in artificial immunisation not only are nucleins added by injection, but that nuclein-forming organs and tissues may be stimulated to form nuclein. It is an interesting fact that Wooldridge,¹ protected animals from anthrax by injections of 'tissue-fibrinogen,' a proteid substance which he prepared from the thymus as long ago as 1887. It has already been mentioned that Halliburton's 'cell-globulin β ' is bactericidal. These substances and also Wooldridge's 'tissue-fibrinogen' have now been proved to be 'nucleo-albumins,' by Halliburton,² Pekelharing, and Wright.

Kossel³ states that in lymph-cells in a condition of physiological activity free nucleic acid is apparently present. Like the American investigators, he finds that nucleic acid is bactericidal, and the suggestion is made that in phagocytosis, the amœboid cells having been attracted to the point of attack, the invading bacteria are destroyed by nucleins (or their precursors) either secreted by, or formed by the disintegration of, the amœboid cells.

The term 'alexin' has been applied to the proteid substances which occur in the animal body and which may aid in the defence against bacterial attacks.

Acquired immunity may be induced in either of the following ways:—

1. By an attack of the disease ending in recovery.
2. By vaccinating with a modified and less virulent form of the infective agent (Pasteur's method).
3. By one or more treatments with sterilised cultures, or bacteria-free toxins.
4. By injection of the blood serum from an animal treated or immunised by method 3.
5. Occasionally by treatment with sterilised cultures or

¹ *Collected Papers*, p. 339.

² *Loc. cit.* p. 24.

³ *Deut. Med. Woch.* Feb. 1894, p. 146.

toxins of a different species (*Pyocyaneus* protects from anthrax, and Klein's experiments mentioned above).

Various explanations have been given of the production of acquired immunity. Pasteur suggested that the organism, by its growth in the body, exhausted some specific pabulum necessary for its development, so that the organism could not again grow in the animal which had been attacked. This hypothesis, therefore, presupposes that in the body there is some nutrient material necessary for the growth of each species, which it is difficult to believe, and is negatived by the fact that an organism will grow in the blood and tissues removed from an animal vaccinated against, and insusceptible to, the disease produced by it. Chauveau in his retention theory suggested that the bacteria during their growth in the tissues formed substances which ultimately inhibited their growth, and, if the animal recovered, prevented a subsequent development of the organism.

Some German investigators suggest that each germ produces both toxins and immunising substances. Fränkel, for example, states that if diphtheria or pneumonia cultures be heated to from 55° to 60° C. the toxins are destroyed, but the immunising substances still remain, the latter being destroyed only at a temperature of 70° C.

Ehrlich by his classical experiments with abrin and ricin, two toxic proteids obtained from the jequirity and castor oil beans respectively, showed that acquired immunity is of two kinds, one, 'active,' as he termed it, of long duration, and resulting from an attack of the disease or vaccination with a modified virus and not transmissible to the foetus; the other, 'passive immunity,' resulting from the inoculation of an animal with the blood serum derived from another animal immunised by the injection of bacterial toxins. 'Passive immunity' is soon lost, but while present is transmitted to the foetus.

Acquired immunity may be induced by inoculation with increasing quantities of bacterial toxins. The animal finally

acquires a high degree of immunity, which is transmitted to the foetus through the blood of the placental circulation and to the nursing through the milk. The immunising substances, which are produced by inoculating animals with bacterial toxins, have been termed antitoxins by Behring and his co-workers, and Behring has formulated a theory of acquired immunity in which he states that the bacterial toxins are so modified by some property acquired by the refractory animal that they become innocuous.

The manner in which the bacterial toxins are rendered innocuous is not exactly known. It was once thought that the antitoxin directly neutralised the toxin in the same way that an acid neutralises an alkali, an inert compound being formed, but some experiments by Buchner seem to negative this. He injected each of several mice and guinea-pigs with an equal amount of tetanus toxin ($\cdot 0001$ gram). All the mice died, while the guinea-pigs recovered; mice are therefore more susceptible to this dose of toxin than guinea-pigs. He then injected into each of twenty-three mice and a like number of guinea-pigs a solution of $\cdot 014$ gram of tetanus toxin and $\cdot 00135$ gram of tetanus antitoxin, mixed some time previously to allow of neutralisation, if such there be. Each mouse therefore received at least 140 times the fatal dose of toxin. Of the twenty-three mice only three died, eleven exhibited passing tetanic symptoms, and nine were unaffected. If this result had been due to direct neutralisation in the mixture a still better result should have obtained in the case of the guinea-pigs, which by the previous experiment were proved to be less susceptible than the mice to the tetanus toxin. But of the twenty-three guinea-pigs eight died, twelve had severe symptoms, and only three were unaffected.

It seems more likely that the antitoxin acts by rendering the cells and tissues insusceptible to the toxins, though in what manner is quite unknown. Antitoxic serum prepared from toxins only is not germicidal, and the organisms will grow

and multiply in it; but if in addition to toxins the organisms also are injected during its preparation, the antitoxin is not only antitoxic, i.e. antagonistic to the toxins, but is also anti-microbic or germicidal. Immunity conferred by injecting antitoxins is of short duration, lasting not more than about three weeks, after which time the animal becomes again susceptible.

Natural immunity in most cases is certainly not due to the presence of antitoxins; for example, the blood of the fowl, a bird which is highly insusceptible to tetanus, has not the slightest antitoxic power towards a tetanus toxin. Moreover, in the preparation of an antitoxin by injecting an animal with toxins the immunity towards the toxins gradually increases, and *pari passu* the antitoxic power of the serum increases up to a certain point, but then slowly and steadily falls, until it is almost lost, yet the animal remains as highly immune as ever. In a natural disease also, such as enteric fever, while substances of the nature of antitoxins seem to be present in the blood in the early stages and perhaps help to ultimately bring the attack to an end, the immunity, more or less complete, which is enjoyed by the individual for the remainder of life cannot be due to their presence.

It seems, however, that in certain cases the blood serum has some antitoxic action, although the individual has never suffered from the disease, and may serve to render him insusceptible.

Thus Wassermann¹ found the blood of some healthy persons antitoxic as regard diphtheria, and Orłowski² observed the same thing in a few children, a previous attack of the disease having been excluded as far as possible. That such individuals have not necessarily passed through an attack of diphtheria seems proved by the observations of Meade Bolton³

¹ *Deutsch. Med. Wochenschr.* 1894.

² *Ibid.* 1895.

³ *Journ. of Exper. Med.* i. 1896, p. 543.

and of Cobbett,¹ both of whom have met with horses whose blood was definitely antitoxic as regards diphtheria.

The spleen may have some relation to immunity, for experiments by Tizzoni and Cattani seemed to show that rabbits could not be rendered refractory to tetanus by injection of tetanus antitoxin after its extirpation; and although Benario denies this, the manner in which the spleen is attacked in such diseases as tuberculosis, plague, &c. points to this conclusion.

According to Ehrlich, the progeny of an immune father (immunised by the injection of toxins) and a normal mother are not refractory—if anything are more susceptible than control animals; but with immune mothers and susceptible fathers the immunity was transmitted. Some experiments by Tizzoni and Cattani point to the occasional transmission of some degree of immunity towards rabies through the father; such immunity is permanent and contrasts with the transient immunity transmitted through the maternal blood or milk.

To sum up, immunity is probably due to a number of factors, some or all of which may be operative in particular instances, and it is impossible to state with certainty any general law. In some cases phagocytosis is a powerful factor, in others the germicidal action of the fluids of the body; in others, again, the cells and tissues for some unknown reason are unaffected by the bacterial toxins.

Antitoxins and antitoxic treatment, in view of their importance, may here be referred to more in detail.

Antitoxins are prepared by injecting animals, preferably horses, but goats, rabbits, &c. are also employed, with bacterial toxins or living or dead cultures.

With those organisms which produce powerful toxins, such as diphtheria and tetanus, it is customary to grow the organism in a fluid medium so that an active and virulent toxin is

¹ *Journ. of Path. and Bact.* iii. 1895

obtained. The culture is then filtered through a Berkefeld or Pasteur-Chamberland filter and the toxic filtrate inoculated into an animal, generally a horse, commencing with small quantities.

The dose of toxin can be gradually increased, and concurrently with the increase in insusceptibility the blood serum acquires antitoxic properties. The treatment is tedious and the activity of the antitoxic serum is dependent upon the amount and activity of the toxin injected, experimental difficulties alone preventing the preparation of an antitoxin for all those diseases in which the specific micro-organism has been isolated. The requisite degree of strength having been attained, the horse is bled with aseptic precautions, the blood allowed to coagulate, and the serum drawn off and bottled for use. Besides the fluid serum, a dried product is prepared, and a concentrated form obtained by precipitation (Aronson's) may also be had.

Antitoxin, or a substance possessing similar properties, seems to be developed by the electrolysis of diphtheria toxins or of emulsions of bacilli. Smirnow¹ electrolysed diphtheria toxins for sixteen to eighteen hours with a current of 80 milliampères and obtained a product which would immunise and cure like diphtheria antitoxin. Krüger² obtained better results by electrolysing suspensions of the bacilli in 1 per cent. salt solution. D'Arsonval and Charrin³ were unable to obtain antitoxin by the continuous current, but by using an intermittent current some was formed in a quarter of an hour. Meade Bolton and Pease⁴ by using a current of 44 to 72 milliampères for about two hours on broth cultures of diphtheria obtained antitoxin at the positive pole. The strongest was such that 2 c.c. neutralised ten times the

¹ *Berl. Klin. Woch.* 1894, No. 30, and 1895, Nos. 30 and 31.

² *Deutsch. Med. Woch.* 1895, No. 21.

³ *Comp. Rend. Soc. Biol.* 1896, Nos. 3, 4 and 5.

⁴ *Journ. of Exper. Med.* i. 1896, p. 537.

minimal fatal dose. The presence or absence of bacilli did not seem to influence the result.

The antitoxic constituent of antitoxin seems to be a proteid body. Tizzoni by precipitating the antitoxic serum by saturation with magnesium sulphate at 30° C. obtained the antitoxin in the precipitate. Brieger and Boer¹ found that by adding 4 grams of potassium chloride or iodide to 10 c.c. of the antitoxic serum diluted with an equal volume of distilled water and dissolving, then adding 4 to 5 grams of finely powdered sodium chloride and keeping the mixture at 30° to 37° C. for eighteen to twenty hours, the antitoxic constituent was precipitated with a slight admixture of other proteid and salts; the dried precipitate from 10 c.c. of serum weighed 0.4 gram. By precipitating the proteid matter in the serum with a dilute solution of basic lead acetate to which a trace of ammonia had been added, filtering, saturating the filtrate with ammonium sulphate, filtering, re-dissolving the precipitate, dialysing and drying, a light powder was obtained which contained the antitoxic constituent; from 10 c.c. of diphtheria serum 0.06 gram was obtained.

Lastly, by diluting 10 c.c. of the antitoxic serum with five times its volume of water, and adding 20 c.c. of a 1 per cent. solution of zinc sulphate or chloride, Brieger and Boer were able to precipitate the antitoxin as a zinc compound. The precipitate is dissolved in feebly alkaline water (one drop of normal soda solution to 20 c.c. of water) and a stream of carbon dioxide gas passed through the solution. The antitoxin is found in the solution when the original precipitation has been performed with zinc chloride, but is entangled in the precipitate of zinc carbonate when zinc sulphate has been used.

For literature on immunity and antitoxins, see Klein and Buckmaster, *Science Progress*, 1894-97.

¹ *Zeitschr. f. Hyg.* xxi. 1896, p. 259.

CHAPTER VI.

SUPPURATION AND SEPTIC CONDITIONS.

THE subject of suppuration is of great practical importance, and a knowledge of its etiology is one of the main factors which have conduced to the great advances which have been made during the Victorian era in the treatment of wounds, both accidental and those made by the surgeon's knife.

Ogston in 1881 and Rosenbach in 1884 demonstrated that micro-organisms are almost invariably present in the pus of acute abscesses, and these observations have been repeatedly confirmed by subsequent investigators.' A number of experiments were then initiated to ascertain whether these organisms bore a casual relation to the phenomena of suppuration or were merely accidentally present. These experiments show that a large number of organisms can produce suppuration, and render it certain that in ninety-nine cases out of a hundred the suppurative and septic conditions met with spontaneously, or occurring after surgical interference, are due to the action of micro-organisms.

Under the terms of suppuration and septic diseases will be included abscesses, boils, and carbuncles, cellulitis, osteomyelitis, erysipelas, gonorrhœa, ulcerative endocarditis, pyæmia, puerperal fever, and hospital gangrene. It will be convenient first of all to describe the organisms met with in these conditions, and then to discuss the experimental evidence of their relation to the disease processes with which they are associated.

MICROCOCCUS (STAPHYLOCOCCUS) PYOGENES AUREUS.

Morphology and Biology.—A minute spherical organism measuring $\cdot 5 \mu$ in diameter. It occurs generally in more or less irregular groups, but may be met with singly or in pairs (fig. 24). It is non-motile, does not form spores, and stains well with all the anilin dyes and also by Gram's method. It is aërobic or facultative anaërobic, and will grow *in vacuo*. It

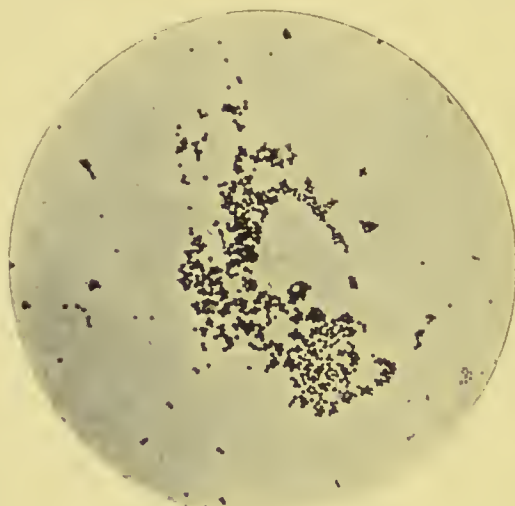


FIG. 24.—STAPHYLOCCUS PYOGENES AUREUS. COVER-GLASS PREPARATION OF A PURE CULTURE. $\times 1000$.

grows well and rapidly on all the usual culture media at temperatures from 18° C. to 37° C. On agar-agar it forms a thickish, moist, shining growth, cream-coloured at first, but after a day or two developing a characteristic orange-yellow colour. It grows in the same manner on blood serum without liquefaction of the medium. Gelatin is rapidly liquefied, the liquefied gelatin being at first somewhat turbid from yellowish masses of organisms; these later on subside and form an orange-yellow sediment (fig. 25). In gelatin plates the colonies form

at first small whitish, granular points, developing in two or three days into circular areas of liquefaction with yellowish masses of the organism floating in them. On potato it forms a similar growth to that on agar. When grown in milk it produces coagulation. Acid production (lactic and butyric acids) can be demonstrated by growing on a neutral litmus sugar-agar. When grown in broth or peptone water it gives the indol reaction with the addition of a nitrite, but not without.

The colour production varies; sometimes it is much deeper than at others, and recently isolated cultures show it better than old ones, and the presence of oxygen also seems to be necessary. The amount of acid production appears to vary directly with the virulence.

In a case of ulcerative endocarditis examined by Sidney Martin,¹ in which the infective agent was the *staphylococcus pyogenes aureus*, a large amount of an albumose and of a basic body was extracted from the blood and spleen. The albumose produced fever and wasting, and retarded the coagulation of the blood.

Leber extracted a crystalline body, which he termed phlogosin, from cultures of the *S. pyogenes aureus*, and Brieger also obtained a crystalline base.

The decomposition products of the action of the *S. pyogenes aureus* on egg-albumin are, according to Emmer-



FIG. 25.—STAPHYLOCOCCUS PYOGENES AUREUS. STAB CULTURE IN GELATIN ONE WEEK OLD, SHOWING LIQUEFACTION.

¹ 'Chemical Pathology of Diphtheria,' &c., Gulstonian Lectures, *Brit. Med. Journ.* 1892, i. p. 755.

ling,¹ phenol, indol, and skatol, many volatile and non-volatile acids, betaine, and trimethylamine.

The *staphylococcus pyogenes aureus* is by far the commonest of all organisms met with in suppurative processes. Ogston found it alone in thirty-four, and associated with the *streptococcus pyogenes* in sixteen, out of sixty-four cases of abscess. It occurs in acute abscess and boils, in some cases of puerperal fever and ulcerative endocarditis, and is almost invariably found in osteomyelitis, but only occasionally in pyæmia. The organism injected under the skin of man or animals produces an abscess, and injection into the bloodstream under certain conditions is followed by ulcerative endocarditis or pyæmia. Impetigo pustules are produced by inoculation into the skin.

It may be said to be universally present on all parts of the skin, and in the mouth, and is frequently met with in the air. According to Sternberg, recent cultures in gelatin are destroyed by an exposure to from 56° to 58° C. for ten minutes; but when dried much higher temperatures, 90° to 100° C., are required, and in the dried state (on a cover-glass) it retains its vitality for more than ten days. According to different experimenters, from two to ten minutes are required to destroy it with a 1-1000 mercuric chloride solution; but it is evident that much depends on the state of aggregation of the organisms, and Abbott has shown that while most of the cocci in a culture are destroyed in five minutes, a few may survive much longer.

MICROCOCCUS PYOGENES ALBUS, AND M. PYOGENES CITREUS.

These organisms are of far more rare occurrence than the preceding one. In morphology and cultural characteristics they agree with the *staphylococcus pyogenes aureus*,

¹ Ber. der Deutsch. Chem. Gesellsch. 1896, 29, p. 2721.

except that the *albus* produces a white, shining, porcelain-like growth, and the *citreus* a lemon-yellow growth. They are said to be less pathogenic than the *aureus*, and are only occasionally found alone, being usually associated with the *aureus*. Cheyne, however, states that in his experience the *albus* is more virulent than the *aureus*, and mixed infections with the *aureus* are regarded as more severe than infection with the *aureus* alone. The *albus* has been found in some cases of panophthalmitis, and is said by Flügge to be commoner than the *aureus* in the lower animals. A white staphylococcus, the *staphylococcus epidermidis albus* of Welch, is very common on the skin, and is probably merely a variety of the *staphylococcus pyogenes albus*, and appears to be frequently present in wounds healing normally.

STREPTOCOCCUS PYOGENES.

Next to the *staphylococcus pyogenes aureus*, this organism is of the greatest practical importance.

Morphology.—Minute non-motile, spherical cocci measuring about $1\ \mu$ in diameter, but varying considerably in cultivations.

Multiplication is by fission in one direction only, so that chains of cocci are formed. In pus these chains do not usually contain more than ten to fifteen elements (fig. 26), but under cultivation, and especially in broth cultures, they may be much longer, and occasionally in a chain an irregular division occurs, so that a branch chain forms. A cell here and there in a chain is often somewhat larger than its fellows, and some authors have considered these enlarged individuals to be arthrospores.

It can be cultivated on the usual culture media, and grows anaërobically as well as aërobically. On agar, or better glycerin agar, it forms in twenty-four to forty-eight hours small whitish, semi-transparent, more or less isolated

colonies (fig. 27). On gelatin the growth has much the same characters, but is better seen, as this medium is clearer than agar, and it takes some days to attain the maximum. In stab-cultures it forms minute spherical colonies all down the line of the stab, but does not invade the surrounding medium. The gelatin is not liquefied. In broth it forms a flocculent deposit, the fluid usually remaining clear. It does not develop on potato, but in milk it grows well, usually, but not always, with coagulation. The indol reaction can be obtained in

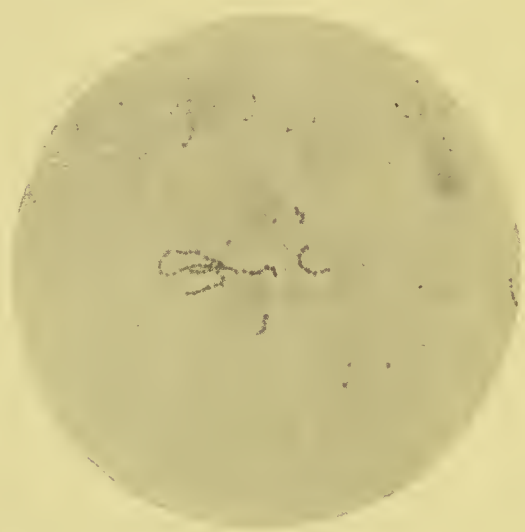


FIG. 26.—STREPTOCOCCUS IN PUS. COVER-GLASS PREPARATION. $\times 1000$.

broth cultures in seven to fourteen days on the addition of a nitrite, but not without. It gives an acid reaction when grown on neutral litmus sugar-agar. It is the only organism with which the writer is acquainted that does not reduce a weak solution of methylene blue.

There are slight differences in the cultural characters and morphology of the streptococcus derived from different sources, and its virulence varies very considerably. Von Lingelsheim has named two varieties, *streptococcus brevis* and

streptococcus longus, the former being feebly pathogenic, the latter markedly so, but they seem to be merely accidental varieties and not true species.

According to Sternberg, the thermal death-point of *streptococcus pyogenes* is 52° to 54° C., the time of exposure being ten minutes. It is destroyed by a two hours' exposure to 1-300 carbolic acid, and 1-2400 mercuric chloride solutions.

The *streptococcus pyogenes* is sometimes found in acute circumscribed abscesses. Zuckermann from an analysis of the results of various observers states that it occurred in 16 per cent. The streptococcus is, however, especially frequent in spreading inflammations, lymphangitis, cellulitis, and progressive gangrene, and is the usual cause of pyæmia and puerperal fever. It is met with in about one-third of the cases of ulcerative endocarditis, occasionally in acute osteomyelitis, and seems to be the cause of the septic pneumonia so often observed in operations about the mouth and throat.

In erysipelas, streptococci are present in the lymphatics at the margin of the zone of redness. These were first isolated by Fehleisen, who described the organism as the *streptococcus erysipelatos*, and by inoculation experiments on man and animals demonstrated its causal relation to the disease. The experiments on man were made in cases of extensive and inoperable carcinoma and sarcoma, as it had

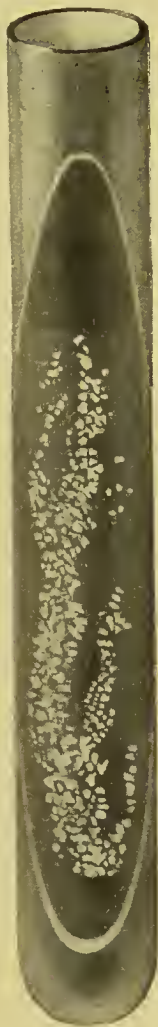


FIG. 27. - STREPTOCOCCUS PYOGENES. GLYCERIN AGAR CULTURE.

been noticed that malignant tumours were frequently benefited after an attack of erysipelas. Several cases were inoculated, and all were successful with one exception, typical erysipelas developing. At one time the *streptococcus erysipelatos* was considered to be different from the *streptococcus pyogenes*, but the two organisms are now regarded as identical, the differences in cultural characters being slight and not constant.

Petruschky ¹ has recently produced a typical erysipelas in the human subject by inoculation with a pure culture of a streptococcus derived from a case of suppurative peritonitis, and Bulloch ² has shown that an animal immunised against a streptococcus derived from a case of erysipelas is also immune against a streptococcus isolated from an abscess.

At the same time, clinically, the streptococci seem to 'breed true,' i.e. the streptococcus from a case of erysipelas introduced into a wound produces erysipelas and not cellulitis.

The different effects produced by the *streptococcus pyogenes*, abscess in one case, erysipelas in another, cellulitis or pyæmia in a third, are probably attributable partly to differences in virulence and partly to the seat and mode of entrance into the body. Streptococci have been described in a number of diseases about which we know little, such as variola, scarlatina, and vaccinia, but it is doubtful whether they bear any causal relation to these conditions. Strangles, a disease of horses, seems to be due to them.

The important lesions due to the streptococcus and their grave nature have led to the attempt to prepare an antitoxin, but many and great experimental difficulties have to be overcome to do this. The virulence of the streptococcus has to be increased by passing it through a series of rabbits, and it is only by growing it in serum mixtures that satisfactory cultures

¹ *Zeitsch. f. Hyg.* xxiii. 1896, p. 142.

² *Trans. Brit. Inst. of Prev. Med.* i. 1897, p. 6.

for the inoculation of the horses can be prepared. Human serum is the best, but is difficult to obtain ; a mixture of asses' serum and peptone beef-broth comes next. The cultures are grown for about a fortnight and are then inoculated into horses, the living cultures being used, and after a time the blood acquires antitoxic properties. The streptococcus antitoxin has been employed in erysipelas, cellulitis, puerperal fever and pyæmia, in many cases with apparent success. Cheyne also suggests its use as a prophylactic before operations about the mouth and throat to avoid septic pneumonia.

STAPHYLOCOCCUS CEREUS ALBUS AND STAPHYLOCOCCUS CEREUS FLAVUS.

These organisms were described by Passet as occurring in abscesses. The *albus* forms a white, and the *flavus* a citron-yellow, waxy growth on gelatin, which is not liquefied, a distinction from the *staphylococcus pyogenes aureus* and *albus*.

STAPHYLOCOCCUS FLAVESCENS.

Described by Babes as occupying an intermediate position between *staphylococcus pyogenes aureus* and *albus*, its colour being somewhat golden. It produces abscess, and is perhaps only a variety of *aureus*.

DIPLOCOCCUS PNEUMONIÆ.

This organism is met with in some cases of empyema following pneumonia, and frequently in otitis media, occasionally in purulent arthritis, and in nearly half the cases of purulent meningitis. It is probably the *micrococcus pyogenes tenuis* of Rosenbach, and its characteristics are given at p. 273.

DIPLOCOCCUS INTERCELLULARIS MENINGITIDIS.

This organism, discovered by Weichselbaum, is found in a large proportion (50 per cent.) of cases of cerebro-spinal meningitis. It is a micrococcus usually occurring in pairs, fours, or small masses, and present within the pus cells like the gonococcus. It stains best with Löffler's methylene blue, but does not stain by Gram's method. It does not flourish at room temperature, and hence will not grow on gelatin. On agar at 37° C. it forms a thick, viscid, whitish growth. There is only a scanty growth on blood serum and in broth, and none on potato. It does not grow anaërobically and does not live longer than a week on artificial media. It is pathogenic for mice and guinea-pigs.

BACILLUS PYOCYANEUS.¹

This is the organism found in green and blue pus, and it also occurs on the surface of the body. Its presence in wounds greatly retards healing, and occasionally a general toxæmia may result from it. It has been met with in otitis media and in the green pus of the pleural and pericardial cavities. It is a slender bacillus measuring 4 to 5 μ , frequently united in pairs and forming filaments. It is actively motile, does not form spores, and is aërobic and facultative anaërobic. On gelatin it grows freely with rapid liquefaction, a greenish, fluorescent colour developing in the liquid, while whitish flocculi of growth sink to the bottom. On agar a whitish, moist layer develops, and the medium is stained a greenish colour. On potato the growth is a dirty brown or sometimes greenish.

Milk is coagulated and a greenish colour develops. Broth becomes turbid, and there is a slight film formation with a

¹ On pyocyaneus infection in man, see *Med. Magazine* v. 1896, Nov. p. 1124 (Symmers).

greenish colour. Oxygen is necessary for the development of the pigment, which is generally a mixture of a blue pigment, pyocyanin, and a yellow one, pyoxanthose. Pyocyanin ($C_{14}H_{14}N_2O$) is said to be an anthracine derivative; it is soluble in chloroform, and on oxidation yields pyoxanthose.

Subcutaneous inoculations of a small amount of a culture produce local abscesses, larger amounts cause œdema, with purulent infiltration of the tissues and death. Animals can be vaccinated by means of small quantities of living cultures or by sterilised cultures. Sterilised cultures will prevent infection (experimentally) by anthrax if used early—that is to say, if an animal be inoculated with anthrax, and shortly afterwards injected with a broth culture of the *bacillus pyocyaneus*, a fatal result is averted.

Williams and Cameron¹ describe four cases of diarrhœa with green stools, wasting, and death, in infants, in which the *B. pyocyaneus* was obtained, and suggest that many cases of marasmus may be due to it.

Gessard describes two varieties, which he terms α and β , of the *bacillus pyocyaneus*.

MICROCOCCUS TETRAGONUS.

This is an organism frequently met with in phthical cavities and expectorated with the sputum, and has also been found in the pus of acute abscesses. The cells occur singly (diameter $1\ \mu$), in pairs, or in fours, and enclosed within a capsule. It stains with the ordinary anilin dyes and also by Gram's method. On gelatin it develops slowly, with the formation of a thick, white, shining growth without liquefaction. On agar the growth has much the same characters, and on potato is white and viscous. Inoculated into animals a local abscess may form, but usually it produces a fatal result, and the organism is found in the blood and organs.

¹ *Journ. Path. and Bact.* iii. 1896, p. 344. (Refs.)

A few cases of general infection in man have been described.

BACILLUS *ÆROGENES* CAPSULATUS.¹

This is an organism occurring in conditions accompanied by much development of gas in the tissues, as in cases which might be described either as phlegmonous erysipelas or gangrene with subcutaneous emphysema, especially after injuries. It is also met with occasionally in perforative peritonitis and in various septicæmic and pyæmic conditions, in the puerperal state, complicated stricture, &c.

The organism is a non-motile bacillus, variable in size, 3 to 6 μ long, and about as thick as the anthrax bacillus. It occurs singly, in short chains, or in clumps, and occasionally in long threads. It stains well with the ordinary anilin dyes and also by Gram's method. A capsule is present, but spores are not formed. It grows well on all the ordinary culture media, slowly at 20° C., rapidly at blood-heat, but is strictly anaërobic. It forms greyish-white colonies on agar, and gelatin is liquefied. In sugar-broth it produces at first a diffuse cloudiness, but later the fluid becomes clear and a whitish, viscid sediment settles. Milk is coagulated. On potato the growth is almost invisible. There is abundant formation of gas in culture media.

It is pathogenic for guinea-pigs and mice, but not for rabbits.

In some cases of gangrene, with blackening of the affected parts and much emphysema, Dunham² has isolated the *bacillus aerogenes capsulatus*.

¹ For an exhaustive paper on this organism see *Journ. of Experimental Medicine*, i. 1896, p. 5. (Welch and Flexner.)

² *Bull. Johns Hopkins Hosp.* viii. 1897, p. 68.

BACILLUS COLI COMMUNIS.

This is one of the most important organisms of suppurative peritonitis and puerperal fever. It is identical with the *bacillus pyogenes fetidus* of Passet, and is fully described at p. 236.

Other Organisms met with in Suppurative and Septic Diseases.—Several other organisms have been met with in suppurative processes, but are either described in another place or are not of sufficient importance to need further mention.

Such are the *Bacillus typhosus* (p. 227), Friedländer's pneumococcus (p. 278), *Bacillus prodigiosus* and *Proteus vulgaris* (Chap. XXI.), *Micrococcus subflavus* (Flügge).

As will have been gathered from the descriptions of the individual organisms, suppuration may be set up by inoculation with several species, and a number of experiments by various observers, carried out by inunction, subcutaneous inoculation, and inoculation into the serous cavities, and circulation, have conclusively proved that this is the case, not only in animals, but also in man.

A problem of great importance is whether micro-organisms are usually the cause of suppuration, or whether mechanical injury, chemical agents, &c. can also produce it. Mechanical injury alone does not seem to be capable of inducing pus production, but it is different with regard to chemical agents. For a long time considerable difference of opinion existed and discordant results were published. These discrepancies have now been to a large extent cleared up, and are found to depend upon the method of experiment and the particular animal and chemical agent employed. That chemical agents should produce suppuration was only to be expected, for it would be against analogy, derived from all other bacterial diseases, if the pyogenic organisms did not produce suppuration through the chemical substances formed by them, and if

these chemical substances acted thus, why should not other chemical substances be found to act in a similar way?

In experiments with chemical agents the greatest care has to be taken to exclude the entrance of micro-organisms. This is best done by sealing the sterilised substance in sterilised fusiform glass tubes and introducing these under the skin or into the tissues with strict antiseptic precautions. When the wounds have completely healed the tubes are broken by pressure and their contents allowed to diffuse into the surrounding tissues.

Sterilised cultures of the *staphylococcus pyogenes aureus* and a crystalline body, phlogosin, obtained by Leber from its cultures, produce abscesses on inoculation. Mercury produces suppuration in the dog, but not in the rabbit; silver nitrate (5 per cent. solution) has a similar action. Ammonia fails to produce pus; it is either absorbed without damage, or if in stronger solution produces necrosis of the tissues. Turpentine produces large sterile abscesses in carnivora, and Brieger's cadaverine is likewise stated to set up suppuration.

Buchner was also able, by warming various bacteria with 0.5 per cent. caustic potash, to obtain a solution containing proteid which was powerfully pyogenic, and Nannotti found that sterilised pus had a similar property. It thus seems certain that a number of chemical substances can set up suppuration. At the same time it must be clearly recognised that suppuration and suppurative complications, as they occur naturally, are to be regarded as due to the activity of micro-organisms in almost every instance.

The mere presence of micro-organisms is not everything, however, for they may be present without producing suppuration; and the same organism, for example the *streptococcus pyogenes*, may at one time produce a localised abscess, at another diffuse cellulitis, and at a third a pyæmia; a number of conditions and factors control and modify the occurrence and the particular form of suppurative disease.

As already alluded to (p. 126), many micro-organisms when injected into the blood-stream are rapidly got rid of; so when moderate quantities of the *staphylococcus pyogenes aureus* are injected into the circulation of a rabbit, abscesses as a rule form only in the kidney. If, however, the organisms be attached to gross particles, so that they cannot pass through the capillaries, embolism occurs and abscesses form about the embolic foci. The effect of inflammation and injury in making a part 'susceptible' is also very marked. Inject the *aureus* into animals in which the endocardium or a bone has been damaged, and in all probability an endocarditis or osteomyelitis will ensue. The dose and concentration of the organisms are also important factors. Watson Cheyne found that by injecting the *aureus* into the muscles of a rabbit, 250,000,000 produced a circumscribed abscess, but 100,000,000 caused its death. So, probably, while the cells in a healthy wound will get rid of a few organisms, if the latter are abundant or in masses they will gain the mastery.

CLINICAL EXAMINATION.

In many cases some idea can probably be formed as to the organisms likely to be present in the pus or discharge, &c. from the clinical characters of the disease, in which case the examination may be more particularly directed towards the isolation of the suspected organism. For example, in a urethral discharge the gonococcus would be specially looked for, in an empyema following pneumonia the *diplococcus pneumoniae*, and in a tropical abscess of the liver the *amaba coli*. In all cases the pus or discharge should be collected with aseptic precautions in sterile capillary pipettes or in sterile test-tubes at the time of operation. The discharge from opened abscesses and from wounds is liable to become contaminated and the original infection to be masked. In septic wounds the infection may be a mixed one.

In all cases the examination should be commenced as early as possible.

1. Make several cover-glass specimens from the pus or discharge.

2. Stain one or two of these with Löffler's blue and one or two with gentian violet or carbol-fuchsin, with or without clearing in dilute spirit. Mount and examine microscopically.

(a) If staphylococci only are detected the presence of the ordinary pyogenic cocci may be suspected. Proceed as in 3, 4, and 5.

(b) If encapsuled diplococci are detected suspect the presence of the *diplococcus pneumoniae* and proceed as in 5 and 7.

(c) If diplococci and tetrads are present note whether they are in groups within the pus cells ; if so suspect the presence of either the gonococcus or *diplococcus intercellularis meningitidis*, and proceed as in 6.

(d) If free tetrads are detected suspect the presence of the *micrococcus tetragonus* and proceed as in 3 and 4.

(e) If streptococci are present the *streptococcus pyogenes* is probably the species. Proceed as in 3, 4, and 5.

(f) If bacilli are present they may be the colon bacillus, the *bacillus aerogenes capsulatus*, the bacillus of malignant œdema, the tetanus bacillus, the typhoid bacillus, the *bacillus pyocyaneus*, or putrefactive bacilli of the proteus group. The clinical history of the case will be a guide what to look for.

a. The colon bacillus, especially frequent in suppurative peritonitis and in diseases of the urinary organs. See page 236.

β. The *bacillus aerogenes capsulatus*, especially met with in foul wounds and gangrenous conditions, with much development of gas.

γ. The bacillus of malignant œdema occurs in septic wounds with septicæmic complications. See page 303.

δ. The tetanus bacillus is found in the wound in cases of traumatic tetanus. See page 302.

ε. The typhoid bacillus is rare ; it may occur in suppurative conditions complicating or following typhoid fever. Proceed as in 3 and 4.

ζ. When the *bacillus pyocyaneus* is present the pus or discharge will probably be blue. Proceed as in 3 and 4.

(g) If yellow granules, having a rosette-like structure micro-

scopically, are present actinomycosis may be suspected and more particularly examined for by the methods given at page 290.

(h) If a mixture of organisms be present, agar and gelatin plate cultivations should be prepared and further examined by subcultures from the colonies.

(i) If no organisms can be detected microscopically, proceed as in 3, 7, or 9. In the pus of ordinary abscesses micro-organisms can generally be detected, unless it be due to the tubercle or glanders bacillus, or to the pneumococcus, or in tropical abscess of the liver.

3. Make several cultivations on agar and gelatin (anaërobic if required), and examine microscopically and by subcultures when the growths have developed.

4. Make two or three sets of agar and of gelatin plate cultivations. Examine the colonies microscopically and by subcultures.

5. Stain two or three of the cover-glass preparations by Gram's method, and counter-stain with Bismarck brown.

6. The gonococcus and *diplococcus intercellularis meningitidis* may be identified and distinguished by the methods detailed at pages 148 and 138.

7. Inoculate guinea-pigs or mice subcutaneously and intraperitoneally with the material.

8. Organisms are rarely detected in the blood; if they be present it is generally a grave sign. They cannot usually be found microscopically, and cultivations must be made.

9. If the abscess be probably a tropical abscess of the liver, the pus or scrapings from the wall of the abscess should be examined for the presence of the *amæba coli*. (Chap. XVIII.)

MICROCOCCUS GONORRHOÆÆ.¹

This organism, although always termed a micrococcus, divides in two directions at right angles to each other, and so would be included in the group 'merismopedia' of Zopf's classification.

¹ For information in this section I am much indebted to Foulerton's monograph in the *Trans. Brit. Inst. of Prev. Med.* i. 1897, p. 39.

It was discovered by Neisser in 1879 in cases of gonorrhœal urethritis. In gonorrhœal pus it occurs usually in pairs, occasionally in tetrads, the elements of which are somewhat ovoid in shape, their opposed surfaces being flattened. The organism has a characteristic arrangement: it occurs in groups *within* the pus cells (fig. 28). The individual cocci vary somewhat in size, the average being about $0.7\ \mu$ in the long and $0.5\ \mu$ in the short diameter. A

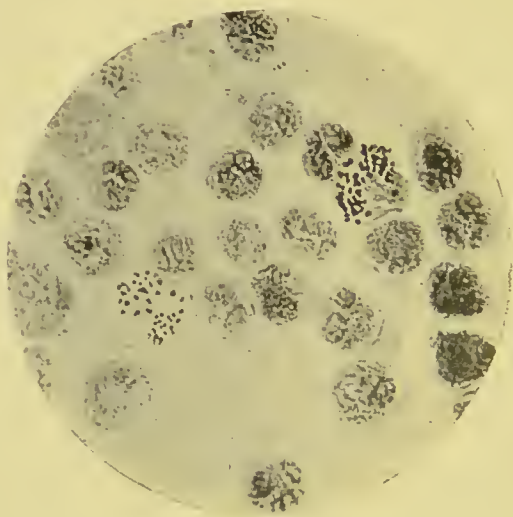


FIG. 28.—THE GONOCOCCUS. COVER-GLASS PREPARATION OF GONORRHOEAL PUS. $\times 750$.

capsule is said to be present, but is difficult to make out except in cultures. It is also said to be slightly motile. It stains readily with the ordinary anilin dyes, Löffler's blue being perhaps the best, but is decolourised by Gram's method—an important practical distinction from the other pyogenic cocci.

The gonococcus is a strictly parasitic organism. It is difficult to cultivate, and usually soon dies out under cultivation—within a week, unless transferred to fresh soil—but it

does not seem to lose its virulence. Growth takes place between 25°C . and 38°C ., but the optimum temperature is 35°C . to 37°C . It is aërobic, and possibly facultative anaërobic, and will grow on a feebly alkaline or acid soil. The ordinary agar and gelatin media are useless for the cultivation of the gonococcus; it will grow only on a medium containing proteid. Blood-serum agar gives fair results, but the ordinary Löffler's blood serum is of no use. Turro employed an acid gelatin, but probably cultivated another organism and not the gonococcus. The best method is to use agar plates smeared with blood. Small Petri dishes are employed, the agar melted, poured into the plates, and allowed to solidify.

Blood obtained by pricking the finger, with antiseptic precautions, is taken up in a sterile capillary tube, and deposited on the centre of each plate. A trace of gonorrhœal pus, collected with aseptic precautions, is taken up on a small sterile camel's-hair brush, and rubbed up with the drop of blood and smeared over the surface of the agar. The plates are incubated at 37°C . and in twenty-four hours the colonies of the gonococci appear as transparent greyish specks, which increase in size up to the end of three days. At this stage the colony measures 1 to 2 mm. in diameter, is raised, brownish and finely granular in appearance, and roundish with a crinkled margin. The cocci from cultures resemble those in pus, but tetrads are more frequently met with. The specific virulence of gonorrhœal pus is destroyed by exposure to a temperature of 60°C . for ten minutes.

The gonococcus is not only strictly parasitic but exclusively affects man, positive results from the inoculation of animals being doubtful.

From inoculation experiments on the human subject it appears to be the specific organism of gonorrhœal urethritis and vulvitis. In the female it is most frequent in the urethral or vulvar discharge, less so in that from the cervical canal, and

rarely or never seen in a purely vaginal one. It is generally, even at an early stage, associated with other organisms, of which Foulerton gives a list of no less than eighteen species belonging to the coccus group alone. These include several diplococci which have to be distinguished from the gonococcus. To identify the latter organism its shape and size have to be taken into account, and two other features will serve to avoid all mistakes, viz. its non-staining by Gram's method and its arrangement in *groups within* the pus cells.

The gonococcus is associated with a variety of lesions besides those already mentioned, viz. epididymitis, ovaritis, and salpingitis, cystitis, peritonitis, arthritis, and conjunctivitis. It has been met with in the blood once, and occasionally produces endocarditis and pericarditis.

Christmas¹ has recently found that the blood serum of the rabbit, fluid or coagulated, is an excellent culture medium for the gonococcus. By cultivating the gonococcus for ten days in an ascitic bouillon mixture he has succeeded in obtaining a toxin which when injected intravenously into rabbits in large doses causes death, in smaller doses fever and loss of weight, while precipitated with alcohol and injected into the anterior chamber of the eye it produces severe inflammation. By injecting rabbits with small doses of the toxin, immunisation is produced, and the blood acquires antitoxic properties.

CLINICAL DIAGNOSIS.

The diagnosis of gonorrhœa is very important both from the clinical and from the medico-legal point of view. For this purpose microscopical examination and culture methods are made use of.

1. *Microscopical Examination.*—Several cover-glass specimens of the pus or discharge should be prepared by smearing a thin film, drying, and fixing in the ordinary way. If the best results are desired, the cover-glasses after smearing are air dried and then fixed by placing in a mixture of equal parts of alcohol and ether

¹ *Ann. de l'Inst. Pasteur*, xi. 1897, p. 609.

for fifteen minutes. After fixing, a couple of the preparations are stained in Löffler's blue for three to five minutes, washed in water, dried and mounted. They are then examined with a $\frac{1}{2}$ in. oil immersion; a lower power lens is no use. The ovoid cocci in pairs, and occasionally in tetrads, occurring within the pus cells in groups of not less than four pairs, are practically characteristic. Diplococci situated outside the pus cells should be neglected. The next step to take is to ascertain the staining reaction by Gram's method. Stain two more cover-glass specimens for fifteen minutes in anilin gentian violet, dip in water, place in Gram's iodine solution for two minutes, decolourise in absolute alcohol until the drainings fail to stain white filter-paper, and counter-stain for forty-five seconds in a saturated aqueous solution of Bismarck brown diluted with three times its volume of distilled water. The gonococci are decolourised and take up the brown stain.

2. *Culture Methods*.—Whenever a diagnosis is of great importance, an attempt should be made to cultivate the organism. Plate cultures of agar smeared with blood should be prepared as described above, and another set of agar only, and incubated at 37°C. In forty-eight hours colonies of the gonococcus should be recognisable on the blood agar, but not on the plain agar.

The *micrococcus gonorrhææ* is the only organism which possesses all the three following characters:—

(a) Occurrence in cell-colonies in pus, (b) decolourisation when treated by Gram's method, (c) inability to grow at blood and room temperature on agar (Foulerton).

The only other organism occurring in groups *within* the pus cells and decolourised by Gram's method is the *diplococcus intercellularis meningitidis* of Weichselbaum, but it grows freely on agar at blood-heat, and is not likely to prove a source of confusion in the diagnosis of gonorrhæa.

SARCINA VENTRICULI.

An organism occurring in the contents of the stomach, especially in cases of dilated stomach. Originally described by Goodsir in 1842.

It occurs as a large ovoid cell, several of which are grouped

together quadrilaterally so as to form more or less cubical masses, the so-called 'woolpacks.' According to Falkenheim it forms on gelatin in thirty-six to forty-eight hours roundish, prominent colonies of a yellowish colour. In neutral hay infusion it forms a brownish film and flocculi. It produces an acid reaction.

Other sarcinæ also occur in the stomach.

CLINICAL EXAMINATION.

1. The organism can be detected in the vomit, &c. most readily by examination in the fresh state, a little of the material being placed on a slide, diluted with water if necessary, covered with a cover-glass, and examined.
2. Cover-glass preparations may be stained with weak carbol fuchsin, or by Gram's method.

For further information on suppuration, &c., see *Suppuration and Septic Diseases*, W. Cheyne; *Traumatic Infection*, Lockwood; and Croonian Lectures by Burdon Sanderson, *Brit. Med. Journ.* 1891, II. p. 983 *et. seq.*

CHAPTER VII.

ANTHRAX.

THE disease known as anthrax claims attention not only on account of its importance, but also from historical considerations. It is, perhaps, the first disease which was definitely associated with a specific micro-parasite, for the anthrax bacillus was observed in the blood of infected animals as long ago as 1849 by Pollender, and in 1850 by Davaine, and the latter also claimed in 1863 to have demonstrated by inoculation experiments the causal relation of the bacillus to this disease. Davaine's experiments, however, cannot be said to have been conclusive, inasmuch as he had not complied with the second and third of Koch's postulates, which declare that the micro-organism must be cultivated outside the body, and the cultivated organism must produce the disease on inoculation. The experiments of Davaine were made by inoculating an animal directly with the blood from an infected animal, and the objection was therefore raised that infection was due not to the bacillus, but to something else in the blood. This objection was subsequently removed by the work of Pasteur and Koch, who obtained pure cultures of the organism, and with them produced the same results as had been obtained by the inoculation with the blood of an infected animal.

Morphology.—The *bacillus anthracis* is a rod-shaped organism varying slightly in size in different animals and under cultivation; in the blood it measures from 5 to 20 μ in length and 1 to 1.25 μ in breadth (fig. 29), but in cultures much longer filaments develop. Examined in the fresh and living

condition in a hanging-drop preparation, these rods and filaments appear homogeneous or slightly granular; in stained preparations, however, they are seen to be made up of a series of segments with unstained interspaces, and the ends of the segments appear cut off square, provided care has been taken not to overheat in fixing and to stain with an aqueous solution. In the blood the filaments never exceed about five or six segments in length, except perhaps in swine, in which animals

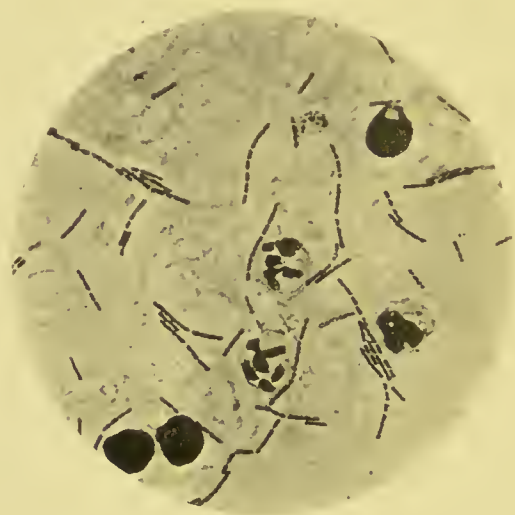


FIG. 29.—ANTHRAX. COVER-GLASS PREPARATION OF BLOOD OF INOCULATED GUINEA-PIG. $\times 750$.

they may be somewhat longer. In cultivations, however, the filaments may be of almost unlimited length and lie parallel to each other or in more or less tangled masses. In the animal body during life, and for some hours after death, spores never occur; but in cultivations more than a day or so old, and from which oxygen has not been excluded, they are always present, almost every segment containing one. The anthrax bacillus is aërobic and facultative anaërobic; it is non-motile, and stains well with the ordinary aniline dyes, and

especially so by Gram's method. It grows readily on all the culture media at from 20°C . to 37°C . the latter being the optimum. Development ceases at temperatures below about 15°C . and above 45°C . Small, cream-coloured, granular colonies develop in a gelatin plate in about thirty hours and in two to three days appear as small, roundish, cream-coloured pasty masses in little pits in the gelatin, due to its liquefaction. Microscopically, the colonies are somewhat characteristic; they

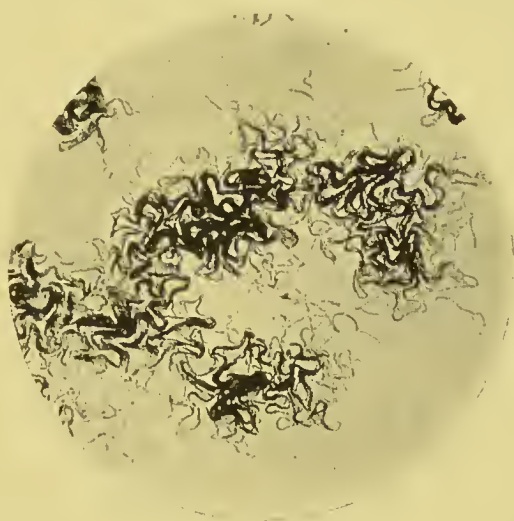


FIG. 30.—ANTHRAX. IMPRESSION PREPARATION OF A COLONY. $\times 40$.

consist of a mass of wavy, tangled filaments like a tiny wad of cotton-wool (figs. 30 and 31). In gelatin streak cultures development is slow, and in four or five days forms a creamy, pasty growth in an area of liquefaction. In a gelatin stab-culture lateral branches spread from the central growth, longer in the upper layers, short below, so that at the end of a week the culture is like an inverted fir tree (fig. 32). This growth develops best in a 5 per cent. gelatin and the gelatin becomes gradually liquefied from above downwards. The colonies on an agar plate develop in twenty hours at 37°C . as

cream-coloured points, which microscopically consist of wavy, tangled filaments similar to those on gelatin. On an agar surface culture at 37° C. there is a copious development in eighteen hours of a thick, cream-coloured, slimy growth, which at this early stage has a finely granular, ground-glass appearance. On blood serum a thick creamy layer forms, with slow liquefaction of the medium. On potato it grows freely, with an abundant formation of spores, as a dry greyish layer. In



FIG. 31.—ANTHRAX. IMPRESSION PREPARATION OF A COLONY. $\times 750$.

broth it forms a somewhat scanty, flocculent deposit, the broth remaining clear and giving the indol reaction.

In old cultures various involution forms are met with; the rods lose their regular shape and become swollen, producing the so-called torula forms, while the homogeneous appearance of the bioplasm changes and becomes granular. Spores are found in all culture media when there has been free access of oxygen, as in surface cultures on potato and agar; but in a deep broth culture, where the supply is limited, spore formation is absent or very scanty. For the same reason spores are never met with until some hours after death, or when the

fluids containing the bacilli come in contact with air, as in the bloody discharge from the nostrils. The life-history of the organism and the development of spores can be well watched in a hanging-drop specimen prepared by inoculating a droplet of broth with the blood of an infected animal. The preparation can be observed on a warm stage, or examined at stated times, being kept in the intervals in the blood-heat incubator. It will be found at the end of twenty-four hours that the short filaments, which alone are present in the blood,



FIG. 32.—ANTHRAX. GELATIN STAB-CULTURE. SEVEN DAYS OLD.

have grown so long that they stretch across the field, while the bioplasm has become granular, and minute shining points are visible here and there. In another twenty-four hours the filaments have extended, the bioplasm has become still more granular, and the shining spots are now well-marked ovoid, highly refractile bodies—the mature spores. In old cultures the rods and filaments are found to have almost disappeared, numbers of spores alone remaining. These spores when placed under favourable conditions of moisture, warmth, and food again produce rods and filaments ;

a little bud appears, which grows in length and ultimately becomes a mature rod, with the empty spore capsule at one end. Sporeless varieties of the anthrax bacillus have been obtained by cultivating it under unfavourable conditions, as at a high temperature (44° C.), or in the presence of minute quantities of antiseptics (1 : 1000 carbolic acid). The spores are of considerable practical importance, for they are highly resistant forms, requiring at least some minutes' boiling for their destruction, whereas the bacilli without spores are destroyed in ten minutes by a temperature of 54° C. The same resistance occurs towards various germicidal substances. While 1 per cent. carbolic acid and 1 : 10,000 corrosive sublimate solutions destroy bacilli without spores quickly, it requires at least 5 per cent. carbolic acid and 1 : 1000 corrosive solutions, acting for not less than an hour, to kill the spores. Anthrax spores will retain their vitality and pathogenic power unimpaired for years in a dried condition.

Hoffa obtained from pure cultures of the *bacillus anthracis* small quantities of a ptomine, which produced fall of temperature and hæmorrhages. Hankin isolated a proteose which in large amounts was fatal, but in small amounts conferred immunity to subsequent inoculation with living bacilli. Brieger and Fränkel obtained a tox-albumin from animals dead of anthrax. Marmier,¹ by growing the anthrax bacillus in a solution of peptone, glycerin, and salts, and subsequent precipitation with ammonium sulphate, obtained a toxin which he states is neither proteid nor basic, and is contained within the bacterial cells.

Sidney Martin,² by growing the anthrax bacillus in alkali albumin for ten days, obtained from the culture albumoses and an alkaloidal body. From the bodies of animals which had died of the disease, chiefly from the spleen and blood, he

¹ *Ann. de l'Inst. Pasteur*, 1895, p. 533.

² 'Chemical Pathology of Diphtheria,' &c., Gulstonian Lectures, *Brit. Med. Journ.* 1892, i. p. 641.

obtained similar substances, the amount of alkaloid being more than double that of albumose. The mixed products produced fever in animals followed by coma and death. The albumose was proved to be the fever, and the alkaloid the coma, producer; the latter also causes a spreading œdema at the seat of inoculation.

The anthrax bacillus is pathogenic for man, cattle, sheep, rabbits, guinea-pigs and mice. The horse and the pig are also susceptible; but adult white rats, dogs, and Algerian sheep are immune. Young white rats or rats fatigued by muscular work can be infected, and frogs and fish, though immune under ordinary conditions, can be rendered susceptible by raising the temperature of their environment. The virulence varies considerably and may be artificially modified in many ways; by passing through a series of susceptible animals it is heightened, by growing in the body of an unsusceptible animal it is lowered, and the latter result is also obtained by cultivating for two to three weeks, at a temperature of 42° to 45° C. or by the addition of certain chemical substances to the culture medium—for example, 0·01 per cent. of potassium bichromate. These methods of ‘attenuation,’ as it is termed, are put to a practical use in the preparation of the anthrax vaccine.

The disease in cattle is known as splenic fever, and though in England only occurring sporadically, or in small outbreaks, in some parts of the world its ravages assume serious proportions; as in Siberia, where it has been termed the Siberian plague, and in France among sheep to such an extent as at one time to threaten the extinction of the sheep industry. By vaccinating the animals, however, the disease has been prevented from spreading; and whereas before vaccination was introduced about 10 per cent. of the animals were lost, the mortality among the vaccinated is only about 0·7 to 0·9 per cent.

Symptoms of the disease in cattle are not very marked.

A beast may appear a little out of sorts and the next day be found dead, or after a day or two's general malaise, fever, and rigors, with a sanguineous discharge from the nostrils and bowel, it dies suddenly. Post-mortem, the chief feature that attracts attention is enlargement of the spleen, which may be two or three times larger than normal, is highly congested, and very soft and friable. Microscopically, the bacillus is found in enormous numbers in the spleen, somewhat less numerous in the blood, and still less so in the liver, kidney, and other organs. Swine do not often suffer from this disease, unless fed with the offal of an infected animal, in which case the chief clinical sign is great enlargement about the throat; this is almost pathognomonic,¹ while the chains of bacilli tend to be somewhat longer than in other animals.

Mice inoculated subcutaneously usually die in about twenty-four hours, and enlargement and congestion of the spleen are very noticeable. An infected guinea-pig dies in from thirty-six to forty-eight hours and usually shows no symptoms until the last, when it may suffer from rigors, with high temperature, convulsions, and staring coat. Post-mortem, the muscular tissue is found to be pale and œdematous, the spleen is enlarged to two or three times its normal size and is highly congested and very soft, and minute hæmorrhages may occur in the serous membranes. Microscopically, bacilli are found throughout the spleen, and are often so numerous that in a stained preparation there appear to be more bacilli than tissue. Large numbers are also present in the blood and lungs, fewer in the liver and kidney; in the latter organ they are almost confined to the glomeruli (fig. 33). Immediately after death, however, comparatively few bacilli may be met with in the blood, the heart, and great vessels.

The spread of the disease in nature seems to result from the ingestion of spores while the animals are feeding. Although the bacillus would be destroyed by the acid gastric

¹ *Journ. of Comp. Path. and Therapeut.* ix. 1896, p. 150.

juice, this is not the case with the spores, which are probably generally developed from the organisms present in the bloody discharges from a stricken animal, and are distributed by wind and flood, and in this way may infect large tracts of pasture. Pasteur suggested that earthworms might bring the spores to the surface in their casts from the buried carcases of infected animals, but some experiments by Koch negative this. The non-sporing bacilli rapidly degenerate and die in a buried carcase.

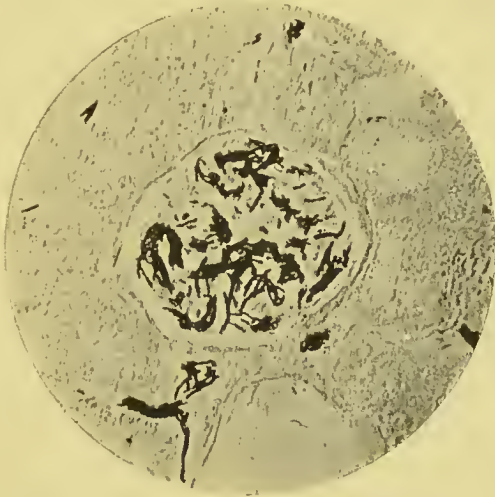


FIG. 33.—ANTHRAX. SECTION OF KIDNEY THROUGH GLOMERULUS. $\times 500$.

In man anthrax is generally met with among those who sort wool or work with hides. The disease occurs in two forms—the so-called ‘malignant pustule,’ a cutaneous infection occurring at the seat of inoculation; and ‘wool-sorter’s disease,’ a general infection through the lungs or stomach. Rag-sorters are also sometimes attacked by anthrax, but there is a ‘rag-sorter’s disease’ which is stated to be due to a motile, non-sporing, non-liquefying, capsulated bacillus, the *proteus hominis capsulatus* of Bordoni Uffreduzzi.

An antitoxic serum for anthrax has been prepared by Marchoux. His method is first of all to immunise sheep or rabbits against anthrax by vaccination by Pasteur's method: they are then inoculated with progressively increasing doses of virulent anthrax cultures, the quantity being doubled every eighth day, until ultimately 200 c.c. to 300 c.c. of a very virulent culture can be given. The treatment has to be prolonged (five months), and the most active serum is obtained between two to three weeks after the last injection. Compared with diphtheria or tetanus antitoxic serums, the anthrax serum is feeble, the most active obtained by Marchoux requiring 0.25 c.c. to protect a rabbit. As a curative agent the anthrax antitoxin was not very successful unless used quite early, and a much more active serum must be obtained before it can rank therapeutically with the anti-diphtheritic and tetanic serums.

CLINICAL EXAMINATION.

1. *In Veterinary Practice.*—If an animal is suspected to have died from splenic fever no extensive post-mortem should be made, because of the distribution of blood, &c. containing the bacilli and the risk of the development of spores, and their subsequent dissemination, with infection of pasture. The abdomen should be opened and the spleen examined. If this be found to be much enlarged, and so soft that it can hardly be handled without rupture, there is a high probability of splenic fever, which the history of sudden death, with or without symptoms coupled with a sanguineous discharge, increases. To confirm the diagnosis, some cover-glass (smear) preparations should be made from the spleen and blood, and can be stained and examined on arriving home. If no cover-glasses are available a small piece of the spleen may be removed, wrapped in a piece of damp rag, placed in a box, so as to avoid any risk of infection, and taken home, where it may be examined.

The cover-glass preparations may be stained with Löffler's blue or by Gram's method with eosin. The characteristic appearances

of these preparations are numbers of large bacilli forming chains of five or six segments, and if the post-mortem has been made within a few hours of death no spores are visible. If a hanging-drop preparation can be made, a notable appearance is the non-motility of the bacilli, which, in the unstained condition, are homogeneous and show no division into segments.

The stained preparations can be kept and produced in a court of law if necessary. Cultivations could also be made from the spleen, but the necessary culture media are not of course usually forthcoming. Finally, a guinea-pig might be inoculated subcutaneously in the abdomen with a particle of the spleen, and on its death examined microscopically and by culture methods.

As regards the disposal of the carcase of an animal dead from anthrax, this should be burned if possible, but, failing this, it may be buried in a deep pit, preferably with plenty of lime. All traces of blood and discharge must be carefully mopped up with a strong lime-wash or solution of chloride of lime, or other reliable disinfectant.

2. *In Man.*—In malignant pustule, cover-glass specimens should be prepared from the fluid of the vesicles or with the scrapings from the incised pustule, or sections of the excised pustule may be made, and stained, some with Löffler's blue, others by Gram's method with eosin. The bacilli are not often met with in the blood. At the same time cultivations on agar and gelatin should be prepared, and may yield positive results when the microscopical examination has been negative.

In all cases of doubt a guinea-pig or mouse should be inoculated subcutaneously with the material, and should the animal die, the diagnosis of anthrax may be confirmed by the characteristic appearances, by a microscopical examination, and by cultivation. The animal experiment is by far the most certain method of diagnosis, a negative result being of nearly as much value as a positive one.

CHAPTER VIII.

DIPHTHERIA.

Diphtheria in England—The Diphtheria Bacillus—The Pseudo-Diphtheria Bacillus—Clinical Diagnosis—The Xerosis Bacillus—Diphtheritic Affections of Birds and Animals.

DIPHTHERIA seems to have been known from the earliest ages and was recognised by the classical (medical) writers, and epidemics of it appeared in England and on the Continent during the Middle Ages. Bretonneau experienced an epidemic at Tours, 1818–1821, and gave to the disease the name ‘Diphtérie,’ from the formation of membranes, which is so marked a feature in it.¹

In England the diphtheria deaths have only been separately scheduled since 1855. Since 1881 there has been a steady increase in the prevalence of diphtheria in London, but not in all England. Formerly this disease was one of rural districts, but now it is distinctly urban. This is well shown in a report by Dr. Longstaff to the Local Government Board in 1887:—

Death Rates from Diphtheria according to the Density of Population.

Period	Sparse	Medium	Dense
1855 to 1860	248	182	123
1861 to 1870	223	164	163
1871 to 1880	132	125	114

In London there has been a marked increase in diphtheria during the last fifteen years, an increase which can hardly be

¹ See *Memoirs on Diphtheria*, New Sydenham Soc. 1859.

explained by a change in diagnosis. For England and Wales the increase is not nearly so marked, as is seen from the following table :—

Mean Annual Rates of Mortality from Diphtheria.

—	1861-70	1871-80	1881-90	1891-95
England and Wales	0·18	0·12	0·16	0·25
London	0·18	0·12	0·26	0·54

Dr. Sykes, in a report to the Vestry of St. Pancras, 1894, has questioned how far the increase in London is real, and has shown, from the Registrar-General's returns, that while the registered mortality from diphtheria has increased, the mortality from other diseases of the throat has *diminished*, indicating that conditions previously registered as other diseases are now returned as diphtheria. The general opinion seems to be, however, that the increase in diphtheria is real and not apparent. Various writers have conclusively shown that a waterlogged condition of the soil, and dampness generally, favour the occurrence of diphtheria, the disease diminishing with drainage.

Diphtheria is distinctly a disease of the young, especially at ages from two to ten, and this holds good both for London and for England and Wales, as the following table shows :—

Death Rates from Diphtheria per 1,000,000, living at various Age-periods

Period 1881-90	All Ages	0-1	1-2	2-3	3-4	4-5	5-10	10-15	15-20	20 and upwards
England and Wales	163	282	685	773	896	848	424	100	36	17
London	260	534	1533	1598	1750	1553	601	96	32	20

It will be seen that diphtheria is especially prevalent at school ages, and Mr. Shirley Murphy, in a report to the London County Council (1894), suggested the importance of 'school influence' in the increase of diphtheria. He showed that the increase was greatest at the age-period three to

ten; that this increase first became conspicuous in 1871, the year after the passing of the Elementary Education Act; and that there is a diminution of diphtheria in London during the summer holidays at the schools. Dr. W. R. Smith, the medical officer to the London School Board, combats these views in a valuable report,¹ and concludes that 'school influence as such plays but an unimportant part in the enormous increase of the disease during recent years in London.'

Personally, from bacteriological evidence, the writer would favour Mr. Shirley Murphy's view, for (1) many cases of diphtheria are so mild as to escape recognition and would tend to spread the disease wherever persons of a susceptible age are congregated, (2) the diphtheria bacilli which cause the disease may remain active in the throat for long periods of time and spread the disease.

Considerable difference of opinion has existed in the past as to the nature of the disease known as diphtheria. At present all are in the main agreed that it is an infective disease, but observers still differ as to what group of clinical characters constitutes diphtheria. The typical cases in which there is a wash-leather-like membrane, leaving a bleeding patch on separation, comparatively low temperature, prostration, and albuminuria, are frequently departed from, and the most experienced physicians are sometimes deceived by cases which run the course of a mild tonsillitis and afterwards develop paralysis. It is safer, and probably more correct, to regard diphtheria as a disease showing marked variations in its clinical manifestations, gravity, and sequelæ, and not to depend on any one symptom or set of symptoms for its diagnosis.

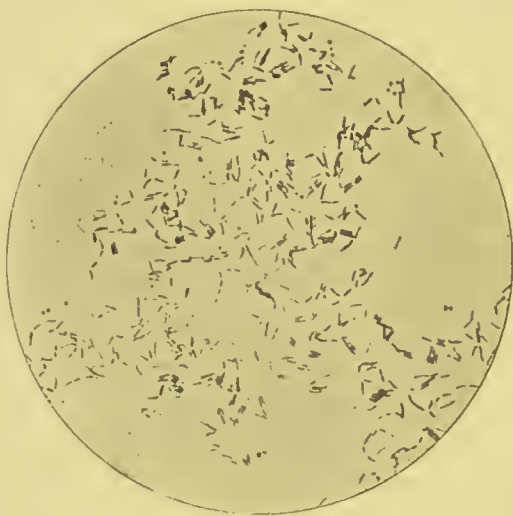
That diphtheria is an infective disease seems amply proved by the history of epidemics, and by the recorded cases

¹ *The Journal of State Medicine*, iv. May 1896, p. 169. The writer is indebted to this Report for the statistical data given above.

where the disease has been conveyed from one individual to another. The bacteriological study of diphtheria was commenced as long ago as 1882 by two German investigators, Klebs and Löffler. Klebs especially investigated the pathological anatomy, and ascribed the disease to small rod-shaped organisms, which he observed to be present in the membrane. It was reserved for Löffler to put this observation of Klebs on a firmer basis by the isolation and cultivation of the bacillus from the membrane, and by the production of certain phases of the disease by the inoculation of the isolated organism. The frequent association of a number of species of bacteria in the membrane made the problem of the isolation of the specific organism by no means an easy matter, and at one time Klein believed that certain micrococci or streptococci, which are frequently present, were the cause of the disease. It is now generally agreed, and the proof seems fairly complete, that diphtheria is dependent upon the rod-shaped organism, the diphtheria bacillus, first described by Klebs and Löffler, and hence frequently termed the Klebs-Löffler bacillus. This organism can be readily isolated from the membrane by the employment of a special culture medium, Löffler's blood serum, which consists of a mixture of blood serum (ox-serum was that originally used), 3 parts, and glucose bouillon, 1 part, the whole being coagulated (see p. 46). On this medium the diphtheria bacillus grows and multiplies exceedingly well, while the other organisms associated with it in the membrane are to a large extent inhibited in their growth. By rubbing a small piece of membrane from a case of diphtheria over the surface of two or three tubes, or of a plate of Löffler's serum, and incubating at 37° C. for twenty to twenty-four hours, colonies of the diphtheria bacillus, more or less isolated according to the number of organisms present in the membrane, will be found, and from these subcultures of the bacillus can be prepared.

CHARACTERS OF THE DIPHTHERIA BACILLUS.

Morphology.—The Klebs-Löffler bacillus is a small delicate bacillus, with rounded ends, measuring 3 to 4 μ in length. The size is somewhat variable even on the same medium, and three varieties of the bacillus have been described, viz. long, medium, and short, according to its length. A few of the rods in the membrane are usually somewhat swollen at one end, the so-called clubbing, and parallel grouping is almost

FIG. 34.—KLEBS-LÖFFLER BACILLUS. $\times 1000$.

universal, the bacilli lying parallel side by side. Thread forms do not often occur, but it is very common for the bacilli to be joined end to end in a series of twos. On different media there are also considerable variations in size. On blood serum and gelatin the bacilli are of medium length; in broth they tend to be short and stunted; while on agar, especially glycerin agar, they are much larger than on the former media. Variations in form are also almost invariably met with on culture media. This is especially marked on

glycerin agar, long club-shaped and spindle-shaped forms being abundant; on blood serum they also occur, but sparsely in a young eighteen to twenty hours' culture, in a forty-eight hours' culture more numerous. It is non-motile, does not form spores, and stains well by Gram's method, and with the ordinary anilin dyes. The most characteristic appearance, perhaps, is obtained by staining with Löffler's methylene blue. The colouration is usually somewhat irregular, the so-called segmentation, more deeply stained portions alternating with paler intervals, and especially marked with agar cultures. The ends of the organisms are also frequently more deeply stained than other parts, the so-called polar staining, while the phenomenon known as 'metachromatism,' is often marked both at the poles and also in the rod, appearing as granules of a purplish tint and contrasting with the blue of the methylene blue.

The diphtheria bacillus grows well on the ordinary culture media, forming cream-coloured growths or colonies—the colonies on serum tending to be somewhat flattened with regular margins. It grows slowly on gelatin, forming a raised whitish growth without liquefaction of the medium, and flourishes in milk, which is not curdled. It generally produces an acid reaction during its growth at first, but afterwards this changes to alkaline. In broth a granular growth usually forms at the sides or bottom of the tube, the broth remaining clear, and there is no film formation; occasionally, however, the broth may become turbid throughout. No gas is developed in the cultures. It is aërobic and also grows well anaërobically. On potato the growth is slight and invisible.

The indol reaction can be obtained in peptone water cultures both with or without a nitrite (see below, p. 181).

As regards the production of acid, Neisser has recently found that during the first nine hours there is little or none; at the end of twenty-four hours a considerable quantity has

been formed, and increases until the end of the second day, after which it ceases.

The Klebs-Löffler bacillus retains its vitality in cultivation for months, and when dried for three or four weeks. According to Welch and Abbott, it is destroyed in ten minutes by a temperature of 58° C. It is readily destroyed by antiseptics when in cultivation, but in the membrane it is difficult to find any agent which will penetrate and kill the bacilli beneath the surface.

The description of the diphtheria bacillus and its characters under cultivation have been somewhat fully gone into on account of the importance of the identification of the organism as a means of clinical diagnosis. As mentioned at the commencement of this chapter, the clinical diagnosis of diphtheria presents many difficulties and considerable assistance may be derived from cultivations from the throat. A piece of membrane or a swabbing from the throat is rubbed over a serum tube, which is then incubated at 37° C. for eighteen to twenty hours, and the growth examined microscopically after staining. The diagnosis is based on the presence or absence of the Klebs-Löffler bacillus. This method is of very real assistance in doubtful, and especially in mild, cases, where clinically it would be very difficult to say whether the condition was diphtheritic or no. The mild cases are those which it is of the greatest importance to identify, especially in schools, for if not recognised the patients may go about and prove a source of infection to all around. The method is also valuable for affording evidence as to when a case can be considered free from infection; as long as bacilli are present in the throat infection must be possible, and it is remarkable how long they may be met with. The average length of time is usually about three weeks, but they may be found much longer. The writer isolated them for as long as five months (and virulent to the last); and a case is recorded in which they persisted for no less than fifteen months after the

attack. In such cases, two or three examinations should be made at short intervals with negative results before the bacilli can be pronounced to be absent. When they persist, treatment with antiseptic sprays or gargles, especially an aqueous solution of 1-2000 corrosive sublimate, combined with syringing the nose, usually soon causes their disappearance. Syringing the nose is important, for the bacilli probably extend to the post-nasal space, where they are untouched by a throat spray or gargle.

With regard to the value to be attached to the bacteriological examination for diphtheria, while the finding of the bacilli is proof positive of the diphtheritic nature of the affection, their absence is not of so much value, as various circumstances modify the result. For example, an unskilled person may not happen to touch the right spot with the swab, or from struggling, &c. on the part of the patient even a skilled operator may fail to touch any but a small portion of the mucous membrane, instead of giving a good mop all round, especially where there are no definite patches of membrane. The previous use of antiseptic gargles or paints will in all probability prevent the growth of the bacilli. It sometimes happens that a very mixed growth is obtained in the cultures, and in such cases the Klebs-Löffler bacillus may be missed. Bearing such sources of fallacy in mind, and making due allowances for them, then the negative result of a bacteriological examination has considerable value in those cases which clinically are doubtful. The bacilli from the throat are frequently associated with other organisms, especially staphylococci and torulæ; and those cases in which the temperature tends to be high and the throat foetid are usually a mixed infection of diphtheria bacilli with the *streptococcus pyogenes* or *staphylococcus pyogenes aureus*. The fact of such mixed infection cannot, however, be definitely decided from the cultures, as these organisms may be present in the mouth or throat without

necessarily taking part in the infective process. Nor can the severity of the disease be gauged from the characters or numbers of the diphtheria bacilli and other organisms present, though perhaps, in a number of cases, those which yield pure or almost pure cultures of bacilli will probably be more severe than the cases which yield cultures with few bacilli. It has been stated that the long form of the diphtheria bacillus is the most, and the short form the least, virulent, the medium being intermediate, but this is by no means a universal rule.

It is sometimes stated that a microscopical examination, unless controlled by inoculation of the isolated bacteria, is unreliable. Such a statement is extremely misleading. If the bacilli which have been cultivated from a suspicious throat possess all the characters of diphtheria bacilli, inoculation experiments are not needed, and if they were performed with a negative result (i.e. the bacteria were not virulent) would prove nothing, for the bacilli from different parts of a culture from a throat often possess very different degrees of virulence. Occasionally, it is true, even the expert may be in doubt about a particular bacillus, but such cases are rare and probably do not occur more than once in 200 or 300 cases. Here an inoculation experiment might help, but would be of no value if a negative result were obtained. It is absolutely essential in the microscopical examination for diphtheria to use a good lens and sufficient amplification, not less than 800-1000 diameters.

The result of the examination of specimens from 1000 cases of suspected diphtheria by the writer and Nolan¹ was, that in 587 cases the diphtheria bacillus was found, in 409 cases it was not found, and in four instances bacilli were observed the identity of which was doubtful. Thus 58·7 per cent. of the cases were true diphtheria. In 40·9 per cent., or about two-fifths, of the cases the diphtheria bacillus was

¹ *Brit. Med. Journ.* 1896, i. Feb. 1.

not found, and the majority of these were probably not diphtheria. A few may have been diphtheritic, though for some reason or other the bacilli were not found, but the error so introduced would not probably amount to more than 2 or 3 per cent. Granting that the majority of cases sent up for examination were doubtful ones, it is apparent what a large number of non-diphtheritic cases must have been returned as diphtheria in the period before the days of bacteriological examinations.

In 600 of the cases notes were kept as to the organisms present in the cultivations, and are set forth in the following table :—

The following Organisms were present alone or associated with the <i>B. Diphtheriæ</i> :—	Cases in which the Diphtheria Bacillus was present, alone or associated with other Organisms.	Cases in which the Diphtheria Bacillus was absent.
<i>Bacillus diphtheriæ</i> alone .	216	Pseudo-Diphtheria 2
Streptococci	6	32
Micrococci	55	79
Bacilli	19	41
Torulæ	9	1
Sarcinæ	6	2
Streptococci and micrococci	2	23
Micrococci and bacilli .	9	19
Streptococci and bacilli .	1	5
Torulæ and bacilli . .	1	3
Sarcinæ and bacilli . .	0	3
Micrococci (including streptococci) and sarcinæ .	6	8
Micrococci (including streptococci) and torulæ .	4	14
Many forms present together	19	15
	353	247

These results are only approximate, as they are based on the more or less brief examination necessary to determine the presence or absence of the diphtheria bacillus, and no special pains were taken to observe all the organisms which might have been present. It is noteworthy that the diphtheria

bacillus was obtained practically in pure cultivation in no fewer than 216 out of the 353 cases in which it was found. In only six was it associated with the streptococcus alone; but too much stress must not be laid on this point, for in a number of instances the mode of transmission of the specimen was not favourable to the vitality of the streptococcus, and in others it was doubtless overlooked, or perhaps included among micrococci. All the forms of the diphtheria bacillus were met with.

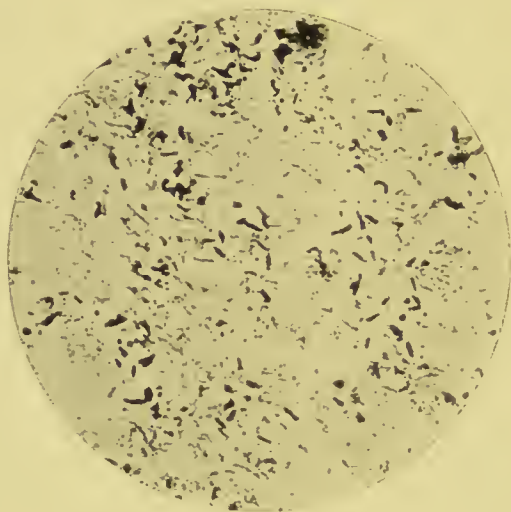


FIG. 35.—DIPHTHERIA MEMBRANE WITH KLEBS-LÖFFLER BACILLI.
GRAM AND EOSIN. $\times 750$.

The diphtheria bacillus is pathogenic for man, the horse, ox, rabbit, guinea-pig, cat, chicken, pigeon and finches, which are all more or less susceptible, while mice and rats are immune. In man the respiratory tract is usually affected, though the conjunctiva and other mucous membranes, as the vagina, and wounds may be attacked. A membrane usually forms, consisting of laminae of fibrin with a few degenerate cells, and here and there small effusions of blood, and in this membrane the bacilli are for the most part located in the super-

ficial layers, though in all cases in which the disease has lasted for any time they are found in the lungs, spleen, and kidneys, and may occur even in the blood. If the patient recovers from the diphtheritic attack paralytic sequelæ are far from uncommon, and are due to a peripheral neuritis. Membranes may probably be formed by other organisms, especially the streptococcus, but it is doubtful whether paralytic sequelæ follow any but a diphtheritic infection.

Another condition which seems to be generally diphtheritic is membranous rhinitis. Whereas true nasal diphtheria is a most serious condition, membranous rhinitis is seldom, if ever, attended with any risk to life, sequelæ do not occur, and a history of infection from cases of it are rare. This is extraordinary and very difficult to explain, for the nose and nasal secretion swarm with virulent diphtheria bacilli.

Guinea-pigs are the animals usually employed for experimental purposes, and in order to compare the effects of various bacilli it is customary to make the inoculation with a measured volume of a forty-eight hours' broth culture. From 0.1 c.c. to 2 c.c. of such a culture, according to the virulence, inoculated subcutaneously, is usually required to kill a 250-gram guinea-pig within forty-eight hours. At the seat of inoculation an extensive hæmorrhagic œdema forms, and hæmorrhages occur in the serous membranes, while the renal epithelium and liver cells undergo cloudy degeneration.

Inoculated into the trachea of the guinea-pig, rabbit, and chicken, pseudo-membranes form, and the same occurs with the superficially injured conjunctiva and vagina. It is stated by some that the diphtheria bacillus does not develop on a normal mucous membrane—this must first be injured, and the staphylococcus and streptococcus, so often associated with the diphtheria bacillus in the human subject, may play a part in preparing the way for infection by damaging the cells and tissues. Rabbits usually live some days or longer after inoculation, and paralysis frequently

develops when life is prolonged, simulating the post-diphtheritic paralysis of man.

Löffler first investigated the chemical products formed by the diphtheria bacillus, and by precipitating bouillon cultures with alcohol obtained a white toxic substance which he classed among the enzymes.

Roux and Yersin precipitated the toxin from filtered broth cultures by means of absolute alcohol, and also by the addition of calcium chloride. They found that 0.4 milligram was sufficient to kill eight guinea-pigs or two rabbits, and considered it to be an enzyme.

From the blood and spleen of cases of diphtheria Sidney Martin isolated albumoses¹ (chiefly deuto-albumose) and an organic acid, but no basic body. Injected subcutaneously the albumose produces much œdema and irregularity of temperature; in larger doses depression of temperature with paralysis and coma. Small multiple doses, not sufficient to destroy life, may give rise to some fever, and in two or three days to paralysis of the hind legs in rabbits, with general weakness and loss of weight. Post-mortem, the nerves were found to have undergone degeneration—breaking up and disappearance of the myelin and interruption of the axis cylinder, while the heart was fatty. The organic acid is also a nerve poison but is not so toxic as the albumose. From diphtheritic membrane, extracted with 10 per cent. salt solution, only traces of albumose and organic acid were found, but the extract was highly toxic, producing fever and paralysis. Martin suggests that a body of the nature of a ferment may be present, and that the ferment in the membrane on absorption, may perhaps form the albumose in the body. From cultures of the diphtheria bacillus in alkali-albumin, albumose and organic acid, with similar actions to those isolated from the body, were obtained.

¹ 'The Chemical Pathology of Diphtheria,' &c., Gulstonian Lectures, *Brit. Med. Journ.* 1892, i. p. 641.

Brieger and Fränkel (1890) were unable to find any basic substance in cultures, and concluded that the toxic substance was a proteid body, which they designated a 'tox-albumin.' It was destroyed by a temperature of 60° C. but not by one of 50° C., even in the presence of an excess of hydrochloric acid, and hence is probably not an enzyme. The tox-albumin is non-dialysable, is precipitated by ammonium sulphate but not by magnesium sulphate, and hence is neither a peptone nor a globulin, contains a large amount of sulphur, and gives the biuret and Millon's tests. A curious property of this body is that small quantities (2·5 mg. per kilogram of the body weight) do not produce their effects until the lapse of weeks. Brieger and Boer in a later research have prepared the diphtheria tox-albumin by precipitating a bouillon culture with a 1 per cent. solution of zinc sulphate or chloride. The precipitate of the zinc double salt is washed with slightly alkaline water and decomposed with a stream of carbonic acid gas. The purified tox-albumin gives the xanthoproteic, biuret, and Adamkiewicz's reactions, and the red colouration on heating with Millon's reagent.

Fränkel in some researches on immunity came to the conclusion that cultures contain two proteid bodies, one of which is poisonous and is destroyed at a temperature of 65° to 70° C., while the other gives immunity and is not destroyed at this temperature.

The question of toxins, and the best means of obtaining toxic cultures, is of importance in the manufacture of a diphtheria antitoxin. This is prepared as follows:—A virulent diphtheria bacillus is grown in such a manner that the cultures attain a maximum toxicity. This is done in various ways, and a number of small points have to be attended to.¹ Slightly alkaline bouillon cultivations are made use of, and free access of oxygen is important. This was formerly attained by passing a stream of sterile air through the cultures. If,

¹ See *Journ. of Exper. Med.* i. 1896, No. 1 (Park and Williams).

however, the bacilli are grown on the surface of the fluid the same result is obtained. This may be done by rubbing up a serum culture with small bits of sterile cork and adding these to the prepared bouillon. The cork of course floats, and the bacilli grow at the surface.

The cultures are grown for about a week at 37°, by which time they attain their maximum toxicity, and are then filtered through a Berkefeld or Pasteur-Chamberland filter to remove the bacilli. The filtrate is germ-free and very toxic, and a little carbolic acid may be added to it to keep it. Selected horses which have been tested with mallein and tuberculin, and kept under observation for some time to see that they are quite healthy, are then inoculated with this filtrate, commencing with a dose of 1 c.c. to 10 c.c. Individual horses vary very much in their susceptibility to the toxin, so that care has to be exercised with the first injections. The injections are given subcutaneously over the shoulder, and produce a local swelling and some rise of temperature and general disturbance, lasting two or three days. When this has passed away the inoculation is repeated, a larger dose being administered provided the reaction due to the former one was not too severe. The treatment is continued, the dose of toxin administered being gradually increased until it may attain 100 to 200 c.c. The greater the reaction which is produced by the first injections, and the more rapidly the dose can be increased, the quicker will an active antitoxin be obtained; but here again horses vary considerably, some yielding a much stronger antitoxin than others under the same treatment. Cartwright-Wood¹ has devised a method for rapidly immunising horses. By growing virulent diphtheria bacilli in ordinary peptone broth, to which an addition of 10 or 20 per cent. of blood serum or plasma has been made, for three or four weeks, subjecting the culture to a temperature of 65° C.

¹ *Proc. Roy. Soc. Lond.* lix. 1896, p. 290. (Also *Brit. Med. Journ.* 1896.)

for an hour and filtration before injection, much larger initial doses can be given and some degree of immunisation attained, and then subsequently the ordinary broth cultures may be injected in large doses.

This method seems to be successful, and is useful in the early stage of injection, as the dose can be more rapidly increased than when an ordinary broth toxin is employed.

The strength of the antitoxin is estimated by ascertaining the amount of antitoxin which will just neutralise ten times the minimum fatal dose of diphtheria toxin in a guinea-pig; this amount is termed a unit. If 1 c.c. of the antitoxic serum is required for this, one unit is contained in 1 c.c.; if 0.01 c.c. is sufficient, then 100 units are contained in the cubic centimetre. In order that the strength of various antitoxins and the work of various standardisers may be comparable, the conditions adopted must be the same. The guinea-pigs must be of the same weight (250 to 260 grams), and the toxin should be of the same strength and kill on the fourth day.

There seems now to be a general consensus of opinion as to the value of antitoxin treatment in diphtheria. The essentials for success are early treatment and the administration of a sufficient amount. Not less than 1500 units should be administered for a dose, and repeated every twelve hours, and in bad cases double or treble this amount should be given. The dose is dependent on the gravity of the disease and not on the age of the patient, and is not necessarily relative to the volume of serum injected, as the serums vary in strength. The first report (1895) on antitoxin treatment in the hospitals of the Metropolitan Board was favourable, and the second report (1896) is still more so. It states that 'the improved results in the diphtheria cases treated during the year 1896 which are indicated by the statistics and clinical observations set forth in the report are:—

‘(1) A great reduction in the mortality of cases brought under treatment on the first three days of illness.

‘(2) The lowering of the combined general mortality to a point below that of any former year.

‘(3) The still more remarkable reduction of the laryngeal cases.

‘(4) The uniform improvement in the results of tracheotomy at each separate hospital.

‘(5) The beneficial effect produced on the clinical course of the disease.’

The advice formerly given as to the paramount importance of commencing the treatment early—if possible not later than the second day of the disease—is repeated.

In addition to antitoxin, local treatment should be pursued as usual, for antitoxin has no direct action on the bacteria which produce the disease.

In cases of mixed infection, where the diphtheria bacilli are associated with streptococci or staphylococci, the diphtheria antitoxin may prove of less value, as it will have no influence on the streptococcic or staphylococcic infection.

The antitoxin has also been employed as a prophylactic as in schools or other places where susceptible individuals are congregated together. For this purpose a dose of about 200 units is sufficient. The practice is stated to have been followed by good results and to have given an immunity to diphtheria, an immunity, however, which does not last more than about three weeks.

The question of the occurrence of the Klebs-Löffler bacillus in the lower animals is of considerable importance with regard to the spread of the disease and the conveyance of infection. The so-called diphtheritic affections of pigeons, poultry, and calves (referred to more in detail below, p. 187) have certainly nothing to do with human diphtheria. A number of observers assert, however, that cats may suffer from the disease, which runs a chronic course, and is associated with bronchitis, lobular pneumonia, nephritis and wasting.

Klein¹ points out that not only are cats liable to the disease in houses where diphtheria has occurred, but that a similar infectious disease exists naturally among cats, and symptoms similar to this natural disease may be produced by inoculating these animals with the Klebs-Löffler bacillus.

Several epidemics of diphtheria seem to have been traced to an infected milk supply. In some instances the infection has undoubtedly been derived from contamination from a human source, but in others this mode of infection could not be demonstrated, and it has been suggested that certain eruptive diseases on the teats and udder of the cow may be caused by the Klebs-Löffler bacillus and the milk become infected therefrom. Klein² has made some experiments with a view of determining this point. He inoculated healthy cows in the shoulder with a bouillon culture of the diphtheria bacillus. This caused fever and local swelling, and in about a week a papular and vesicular eruption appeared on the udders and teats. From the contents of the vesicles the *B. diphtherie* was obtained, and also from the milk on the fifth day, but not subsequently. The cows died in two to four weeks and the *B. diphtherie* was obtained from the local lesions.

Abbott³ obtained somewhat different results in some experiments, but Klein⁴ points out that they were not performed exactly under the same conditions as his own.

THE PSEUDO-DIPHTHERIA BACILLUS.

In connection with diphtheria an important question must be discussed, viz. the occurrence and nature of the so-called pseudo-diphtheria bacillus. The term was originally

¹ 'Report to the Medical Officer of the Local Government Board,' 1889, p. 162.

² '19th and 20th Annual Reports of the Medical Officer of the Local Government Board,' 1889 and 1890.

³ *Journ. Path. and Bact.* ii. 1894, p. 35.

⁴ *Ibid.* p. 428.

used by Löffler, and by the rule of priority should be retained for the organism described by him under this name. There is considerable confusion, however, among authors as to the characters of the pseudo-diphtheria bacillus, and it will be necessary to summarise the literature of the subject. The pseudo-diphtheria bacillus of all authors is a non-virulent organism occurring in the throat in various anginal conditions (scarlet fever, &c.), and occasionally in healthy throats. Parke and Beebe met with it in twenty-seven out of 330 healthy

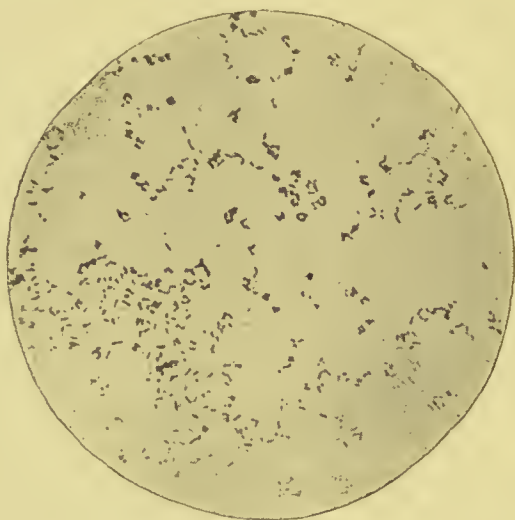


FIG. 36.—PSEUDO-DIPHTHERIA BACILLUS. COVER-GLASS PREPARATION.
× 1000.

throats examined by them. Roux and Yersin, Abbott and Fränkel, describe it as morphologically resembling the Klebs-Löffler bacillus, while Löffler, von Höffmann, Koplick, Parke, and Beebe, Peters, and the author and Miss Knight, consider that an organism differing somewhat from the Klebs-Löffler bacillus should alone be termed the pseudo-diphtheria bacillus, and the following is a description of it from a paper by the latter.¹

¹ *Trans. Brit. Inst. of Prev. Med.* i. 1897.

It is a shortish rod with rounded ends, its average length being from $1\ \mu$ to $1.6\ \mu$, and tending to be slightly thicker at its centre than at the poles. It is non-motile, does not form spores, is arranged in a parallel grouping like the Klebs-Löffler bacillus, and involution forms are, as a rule, not met with. It stains well by Gram's method, and deeply and regularly with Löffler's methylene-blue, segmentation and polar staining being usually absent. On serum, agar, and gelatin it forms cream-coloured colonies or growths, indistinguishable from the Klebs-Löffler bacillus. On ordinary potato it hardly grows at all, what growth there is being quite invisible. On alkaline potato,¹ however, it forms distinct cream-coloured colonies, usually visible by the second day. In stab-cultures in gelatin and sugar-agar no gas is formed, and the growth is confined to the upper part of the stab. In broth it forms sometimes a granular deposit, sometimes a general turbidity. There is no growth anaërobically in hydrogen. On neutral litmus sugar-agar a blue colour is developed, indicating the production of alkalinity. Cultivated in peptone water the indol reaction with sulphuric acid alone can be obtained after a variable time, three to four weeks, while the diphtheria bacillus gives it in about a week; with a nitrite and sulphuric acid the indol reaction can be obtained both with the pseudo- and diphtheria bacilli in about a week. A broth culture reduces a weak solution of methylene blue. It does not curdle milk or liquefy gelatin, can be cultivated at from 22°C. to 37°C. , and is non-pathogenic to guinea-pigs in doses of 5 c.c. of a forty-eight hours' broth culture. The differences between this pseudo-bacillus and the Klebs-Löffler bacillus are shown in the table on the next page.

¹ Ordinary potato rendered alkaline with a 10 per cent. solution of sodium carbonate before sterilisation.

Differences between the Klebs-Löffler and Pseudo-Bacillus.

	Pseudo-Diphtheria Bacillus	Klebs-Löffler Bacillus
Morphology . . .	Rods 1 μ to 1.6 μ in length, tending to be slightly thicker at the centre than at the ends. Is 'plumper,' shorter, and less variable than the Klebs-Löffler bacillus	Rods averaging 1.2 μ to 2 μ in length. Slender and of same diameter throughout. Considerable variation in size
	Involution forms rare	Involution forms usually present
Staining . . .	Stains more deeply and regularly than the Klebs-Löffler bacillus. Polar staining rare	Staining generally more or less irregular, and polar staining common
Alkaline potato .	Distinct cream-coloured colonies or growth visible in two days	Grows well, but growth is almost invisible
Neutral litmus agar	Alkaline reaction	Acid reaction
Stab-cultures in sugar-agar and gelatin	Growth only at upper part of stab	Growth along whole length of stab
Anaërobic cultures in hydrogen	No growth	Grows well
Indol reaction. (Peptone-water cultures, with sulphuric acid alone)	Only after three weeks' growth	After one week's growth

The histories of several cases in which the pseudo was found were obtained, and seemed to show that the pseudo-bacillus is associated with mild anginal conditions, which are free from complications, end in recovery, and are not followed by sequelæ. In many of the cases the anginal condition was associated with distinct patches of membrane, and in two, symptoms were present suggestive of the toxæmia which is met with in diphtheria.

Most authors have been unable to convert the pseudo-diphtheria bacillus into a virulent Klebs-Löffler bacillus or

vice versâ, and are of opinion that it has probably nothing to do with diphtheria (Parke and Beebe, Peters, Washbourne).

In a long series of experiments, however, the writer and Miss Knight obtained some evidence of the conversion of the one form into the other. Moreover, the pseudo-diphtheria bacillus seems in many instances to replace the Klebs-Löffler bacillus in the throat during convalescence, and it is possible to obtain in a large series of cultures connecting links between the Klebs-Löffler bacillus on the one hand, and the pseudo-diphtheria bacillus on the other.

The authors came to the conclusion that in some cases, at least, the pseudo-diphtheria bacillus was a modified Klebs-Löffler bacillus, and the view which they took as to its relation to the Klebs-Löffler bacillus was, that it was a Klebs-Löffler bacillus very far removed from virulence. It would seem, therefore, wise to treat anginal cases in which the pseudo-diphtheria bacillus is found with antiseptic applications and by isolation, though it would probably be inexpedient to admit to a general diphtheria ward, nor would antitoxin be needed in the majority.

CLINICAL DIAGNOSIS.

I. In a minority of cases the diphtheria bacillus can be identified in the membrane and the diagnosis established thereby.

A fragment of the membrane is teased up as finely as possible on a cover-glass or slide, a droplet of water being added if necessary, and a couple of cover-glass specimens prepared. One of these should be stained with Löffler's methylene blue, the other by Gram's method. The bacilli will be found lying parallel to each other in larger or smaller groups, together with involution forms.

II. Frequently the membrane is so crowded with different forms of organisms that it is extremely difficult to recognise the diphtheria bacilli with any degree of certainty. Recourse must then be had to cultivation.

In the writer's experience, no medium is so suitable as Löffler's blood serum, the addition of 10–15 per cent. of glycerin to which, as suggested by Gossage, seems to be an advantage. Kanthack and Stephens have recently devised an agar-serum mixture which they claim is a much better medium than Löffler's serum, but the writer has been unable to satisfy himself of this, while it is much more troublesome to prepare. Agar is not nearly such a suitable medium as blood serum.

A piece of membrane or a swabbing from the throat is rubbed over the surface of one or two serum tubes, care being taken not to break up the medium. The tubes are then incubated at 37° C. for eighteen to twenty hours, and are then examined microscopically whether there is any visible growth or not. If there is no visible growth a scraping is taken by means of a sterilised platinum needle from the whole surface, and a cover-glass specimen prepared with a droplet of water. If there is a visible growth, the cover-glass specimens should be prepared from the most likely colonies, or if the growth be confluent, from the upper half-inch or so. A microscopical examination must always be made, for some colonies—certain staphylococci and torulæ, for example—simulate those of the diphtheria bacillus very closely. The cover-glass specimens should be stained with Löffler's methylene blue for five minutes, washed, dried, and mounted in Canada-balsam or cedar-oil. If there is plenty of growth on the tubes, the preparations may be made on a slide, and after staining, washing, and drying, a drop of cedar-oil may be put on the stained patch, which is then examined directly without a cover-glass. If, however, there is very little growth it is better to make a cover-glass specimen, as the position of the material is so much more easily located. The preparations are examined with a $\frac{1}{2}$ -inch oil immersion magnifying not less than 800–1000 diameters, and the Klebs-Löffler bacillus identified from the description given above.

Löffler's methylene blue gives much more characteristic preparations than Gram's method.

Although eighteen to twenty hours is recommended for incubating the cultures, a microscopical examination will sometimes reveal the bacilli in a much shorter period. The writer has found them in as short a time as six hours.

Neisser has recently recommended the following method of staining :—

(a) One gram of methylene blue (Grübler's) is dissolved in 20 c.c. of 96 per cent. alcohol, which is then mixed with 950 c.c. of distilled water, and 50 c.c. of glacial acetic acid.

(b) Two grams of Vesuvium are dissolved in one litre of boiling distilled water and filtered.

The cover-glass preparations are stained in (a) for one to three seconds, rinsed in water, and stained in (b) for three to five seconds, washed in water, dried, and mounted. Stained in this manner the bacilli are brown, and contain two, or rarely three, but never more, blue corpuscles. The corpuscles are oval, not round, in shape, and their diameter appears greater than that of the bacilli in which they are situated.¹

In the majority of cases, after a little experience, the Klebs-Löffler bacillus will be readily recognised if present. Occasionally, however, bacilli may be present which resemble the Klebs-Löffler very closely, and of which it is difficult to be certain. In such a case the following points should be noted in attempting to come to a decision:—

1. The character of the growth on the medium.
2. The depth of staining with Löffler's blue, and the presence or absence of segmentation or polar staining. The Klebs-Löffler bacillus usually stains pretty deeply, while the bacilli resembling it stain but feebly.
3. The presence or absence of involution forms, clubbing, &c.
4. The presence or absence of thread forms. The Klebs-Löffler bacillus never forms threads.²
5. The presence or absence of spores. The Klebs-Löffler bacillus does not form spores.
6. Motility in a hanging drop. The Klebs-Löffler bacillus is non-motile.
7. Gram's method of staining. The Klebs-Löffler bacillus stains well.

¹ *Zeitschr. f. Hyg.* xxiv. 1897, No. 3, p. 443.

² Klein and others have described thread and branched forms in cultures of the Klebs-Löffler bacillus under certain circumstances, but these are not likely to be observed under the conditions mentioned.

8. The grouping of the organism. The parallel grouping of the Klebs-Löffler bacillus is somewhat characteristic. The bacilli when lying side by side do not seem to quite touch, while the bacilli which resemble the Klebs-Löffler and show a parallel grouping frequently lie much closer together than the Klebs-Löffler bacillus ever does.

If the pseudo-bacillus be found, it is probably wise to regard the case as diphtheritic.

9. The reaction with Neisser's stain. The pseudo-bacillus does not give the diphtheritic reaction.

It occasionally, though very rarely, happens that a conclusion cannot be come to without an extended investigation.

If no serum tubes can be had an egg may be used. It is boiled hard, the shell clipped away from one end with a knife sterilised by heating, and the inoculation made on the exposed white; the egg is then placed, inoculated end down, in a wine-glass of such a size that it rests on the rim and does not touch the bottom. A few drops of water may with advantage be put at the bottom of the glass to keep the egg-white moist. The preparation is kept in a warm place for twenty-four to forty-eight hours and then examined. Antitoxin itself may be used as a culture medium. A test-tube is sterilised by heating, or with boiling water or steam from a kettle, antitoxin to the depth of about an inch is poured in, and is coagulated by holding the tube very obliquely in boiling water or steam. After coagulation and cooling the medium is inoculated. If no incubator is available, the culture may be kept in a warm place, or in an inside pocket.

Many laboratories will now undertake the examination of material. Culture outfits are supplied by some, consisting of a sterilised tube containing a sterilised swab. Failing this, a piece of membrane may be forwarded in a tube or bottle which has been sterilised by heating, or with boiling water or steam. If there be no membrane, a swab can be readily extemporised by wrapping a little wool round the end of a piece of wire, knitting-needle, hair-pin, penholder, or splinter of wood. The wool may be sterilised by moistening with water and then holding in a flame. Membrane or secretion may also be forwarded on pledgets of wool,

pieces of lint or calico, and even on paper, but these are not so suitable.

To detect the diphtheria bacillus in milk, culture methods are unreliable, and recourse should be had to subcutaneous inoculation of guinea-pigs with 0·5 to 1 c.c. of the sediment after centrifugation. A small bacillus can often be cultivated from milk and cheese which, morphologically, closely resembles the diphtheria bacillus.

THE XEROSIS BACILLUS.¹

The xerosis bacillus was isolated by Neisser from cases of xerosis conjunctivæ, and is met with in follicular conjunctivitis. Some say that it occurs in the normal conjunctival secretion, but this is denied by others. In morphology, staining reactions and cultivation characters it resembles the Klebs-Löffler bacillus very closely. It differs from the Klebs-Löffler bacillus in the following particulars: (1) In the *primary* cultivations from the eye on blood serum its colonies do not appear under about thirty-six hours, while those of the Klebs-Löffler bacillus are visible in sixteen to twenty hours. This does not apply to the *secondary* cultivations, in which the colonies appear as soon as those of the Klebs-Löffler bacillus. (2) It does not give rise to an acid reaction. (3) It is non-pathogenic to guinea-pigs.

The part it plays in xerosis, &c., is undecided and its relation to the Klebs-Löffler bacillus is uncertain, *i.e.* whether it be a modified and non-virulent Klebs-Löffler bacillus or a distinct species.

CLINICAL EXAMINATION.

Blood-serum tubes are inoculated with a looped platinum needle from cases of follicular conjunctivitis or xerosis, and incubated at 37° C. for forty to forty-eight hours. Half the tubes will usually show a growth. Cover-glass preparations are made and stained with Löffler's blue and by Gram's method. Subcultures may also be made.

¹ On the xerosis bacillus, see J. Eyre, *Journ. Path. and Bact.* 1896, iv. p. 54.

BACILLUS DIPHTHERIÆ COLUMBARUM.

Pigeon diphtheria, an infectious disease of pigeons, characterised by the formation of diphtheritic-like membranes on the tongue, fauces, and corners of the mouth, was described by Löffler, who isolated a bacillus to which he gave this name. It is short with rounded ends, non-motile, does not form spores, and does not stain by Gram's method. On gelatin it forms a whitish growth without liquefaction, on agar a creamy growth, and on potato a thin grey film. It is pathogenic for the mouse and pigeon, but only slightly so for the fowl and guinea-pig.

Diphtheritic roup of poultry is a different disease and is apparently due to a protozoan parasite.

The so-called diphtheria of calves is produced by an anaërobic streptothrix.

CHAPTER IX.

TUBERCULOSIS—LEPROSY—THE SMEGMA BACILLUS—GLANDERS.

TUBERCULOSIS.

TUBERCULOSIS is, unfortunately, only too common in the human subject, and most of the domestic animals and wild animals in a state of captivity suffer from it more or less. In man we find that the manifestations of tubercle are different at different ages. In the very young general miliary tuberculosis, tubercular meningitis, and tubercular disease of the peritoneum, intestine, and mesenteric glands (*tabes mesenterica*) are the commonest; in older children up to the age of puberty tubercular disease of lymphatic glands, especially in the neck, tubercular disease of joints and bone, and tubercular disease of the skin in the form of lupus are the most common; in young adults, tubercular disease of the lung; and in older people, chronic disease of the lung and tubercular disease of the urinary organs and testes, and of the suprarenal capsules (*Addison's disease*). *Scrofula* and *struma* were terms formerly much employed, but are now little used; both denote a swollen neck and were applied to cases in which there was chronic inflammation and enlargement of the lymphatic glands, the cervical glands being most often affected, while other conditions frequently accompanied them, as inflammation of the ear, throat, and eye, and implication of bones and joints. Such were termed cases of the strumous diathesis, but as just mentioned they are but manifestations of tuberculosis.

The conception of tuberculosis was originally a purely anatomical one, the name being given to a condition in which the organs were studded with little yellowish points or nodules which were termed tubercles. Laennec was the first to point out the characters of these nodules or tubercles, and traced with considerable accuracy their development from beginnings of the size of hemp seed, the miliary tubercles, up to the large cheesy masses which may be met with in the glands and lungs.

Microscopically, a tubercle in its young and typical condition has a more or less definite structure. At the centre one or more large nucleated bioplasmic masses are found, which on account of their size are termed giant-cells, and each containing ten to twenty nuclei situated at the periphery. Outside these is a zone of cells possessing large and distinct nuclei, surrounded by a considerable mass of bioplasm and staining clearly, which, from their resemblance to epithelial cells, are known as epithelioid cells. Outside this again is another zone of smaller cells with scanty bioplasm and small nuclei, known as lymphoid cells from their likeness to the cells of lymphoid tissue. This is the structure of a typical tubercle, but one or other of the component parts may be wanting, and neither can be said to be absolutely characteristic of the tubercle. The nodule possesses no blood vessels, and as its size increases by growth at the periphery the central parts undergo degenerative changes, and may become either structureless or hyaline, or be converted into a soft yellowish material somewhat like cheese and termed caseous. More or less inflammatory reaction is set up in the surrounding tissues by the development of the tubercle, and the cellular elements so produced often become spindle-shaped and ultimately fibrous, so that the tubercle becomes enclosed by a capsule of fibrous tissue which may contract and convert the tubercle into a fibrous nodule. When caseation has occurred calcification may take place—that is, lime salts are

deposited and the nodule is converted into a calcareous mass.

As far back as 1865, Villenin showed that inoculation of rabbits with human caseous material was followed by a development of nodules similar in all respects to the miliary tubercles in man. Cohnheim, Burdon Sanderson, and Wilson Fox confirmed this observation, but they also showed that the development of tubercles apparently followed

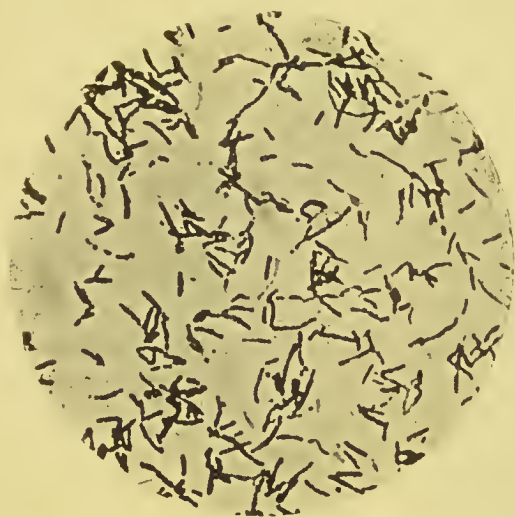


FIG. 37.—TUBERCLE BACILLI, PURE CULTURE. COVER-GLASS PREPARATION.
× 1000.

not only the introduction of tubercular material, but also that of setons, pieces of putrid muscles, and gutta-percha. It was pointed out, however, that in all probability these results were due to accidental contamination or inoculation with tubercular matter, and, by adopting suitable precautions with a view to prevent these sources of error, it has been conclusively shown that non-tubercular matter is unable to set up tuberculosis. Tuberculosis is therefore inoculable, and is an infective disease, and as such must be due to a specific

infective agent, to the discovery of which observers then turned their attention. In 1882 Koch announced that he had discovered a special bacillus in tubercular tissues, which could be isolated and cultivated, and reproduced the disease on inoculation.

The tubercle bacillus (*B. tuberculosis*) is a slender rod with rounded ends, often slightly curved, and measuring 3-5 μ in length. In stained preparations one or more unstained intervals are often seen in the rods (figs. 37 and

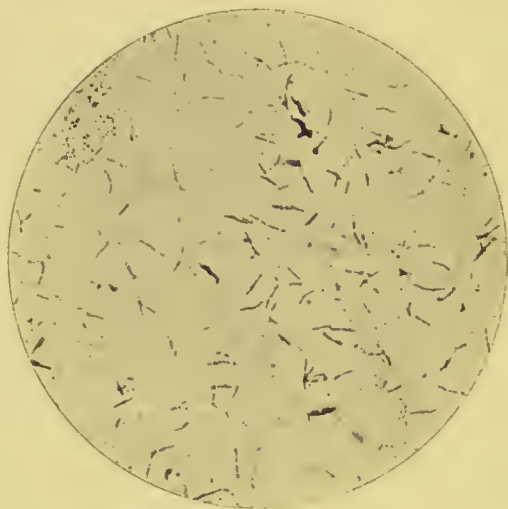


FIG. 38.—TUBERCLE BACILLI IN SPUTUM. $\times 750$.

38); these have been considered by some observers to be spores, but there are many objections to this view. Spores are usually single and not multiple, and are regular spherical or ovoid bodies, whereas the unstained spaces in the tubercle rods are irregular. Moreover, in the same sample of sputum a varying amount of 'beading,' as it is termed, may be brought out by different staining methods. In a specimen stained by Gram's method it is usually more pronounced than in one stained with carbol-fuchsin. In class work also it will be found that one student's specimen will

show beading much more prominently than another's. These considerations render it probable that the beading is an artificial production due to the staining process, and is not a spore formation. The tubercle bacillus, however, probably does form spores, though this is a debated point. Some observers have described clear, regular, unstained spaces in bacilli from old cultivations and consider these to be true spores.

The tubercle bacillus is a non-motile, aërobic and facultative anaërobic, strictly parasitic organism. Usually it occurs singly, occasionally linked in twos or threes so as to form short chains, and under certain conditions, especially in old cultures, filamentous forms have been noticed. It thrives only at a temperature of 37° C. or thereabouts, and its development even then is slow, six weeks at least being required for any appreciable growth. Koch was unable to cultivate this bacillus except on blood serum, but Roux and Nocard have since found that it grows well on nutrient agar or veal broth to which 5 or 6 per cent. of glycerin has been added. The best method of isolating the bacillus from the tissues is to make use of Roux's potato tubes (fig. 8), the bulb being filled with 5 per cent. glycerin. The potato is inoculated with an emulsion of the tuberculous organs and incubated at 37° C. In six or eight weeks cultures will be obtained in half the tubes, whereas if serum or glycerin agar be used not more than 1 or 2 per cent. of the tubes will show a growth. The bacillus will also grow, though very slowly, on glycerin gelatin at 22° C. and on potato. The



FIG. 39.—TUBERCLE BACILLUS.
GLYCERIN AGAR CULTURE
THREE MONTHS OLD.

gelatin and blood serum are not liquefied. On glycerin agar the growth is quite characteristic: it forms a dry, crinkled and wrinkled, cream-coloured or brownish-yellow film, which has been well described as resembling the patches of lichen met with on trees (fig. 39). The growth, however, varies considerably both in colour and in the amount of wrinkling, though retaining more or less the characteristics just mentioned. In broth it forms soft cream-coloured, flocculent masses, which increase slowly both in size and number, the broth remaining perfectly bright and clear. Sometimes a dry crinkled film forms on the surface of the broth, and may spread all over it, and tend to creep up the sides of the vessel. This film formation seems to be essential for the preparation of a satisfactory tuberculin, but it is necessary in order to start it that some of the inoculated particles should float and form nuclei from which the film spreads.



FIG. 40.—FLASK FOR GROWING TUBERCULIN.

The discovery of tuberculin by Koch caused an immense sensation, but unfortunately its rôle as a curative agent has not been sustained.

Tuberculin is prepared by growing the tubercle bacillus in a glycerin veal broth for six to twelve weeks in a shallow layer in flat flasks (fig. 40), so that there is a free supply of oxygen and an abundant growth with copious film formation. The latter seems to be essential, but it does not appear to be important whether the bacilli are virulent or non-virulent.

The cultures, bacilli and all, are concentrated over a water bath to about one-tenth of their volume and then filtered through porous porcelain; the resulting fluid is thick, owing to the concentration of the glycerin by the evaporation, and of a dark amber colour, and possessing a curious characteristic smell. The large proportion of glycerin preserves the fluid, which keeps indefinitely in a cool dark place.

Tuberculin possesses remarkable properties. Injected into a healthy animal or individual it produces no effect, but in a tubercular one minute doses, 0·0003 gram, give rise to a marked reaction—elevation of temperature with constitutional disturbance more or less severe, and swelling and tumefaction of tubercular lesions (glands, ulcers, &c.). By cautiously increasing the dose a toleration is gradually induced, so that large doses cause little or no disturbance. Under certain conditions the injections of tuberculin produce marked changes in the tuberculous parts, leading to necrosis and exfoliation, with subsequent healthy reaction and repair; this is especially seen in cases of lupus. By continued injections a marvellous improvement results, so much so that a cure is apparently effected; but unfortunately when the tuberculin treatment is discontinued the scar usually breaks down and the disease returns. Nevertheless a few cases have remained permanently healed.

Healthy guinea-pigs bear considerable injections of tuberculin without harm; but if they be tubercular, doses of 0·01 gram produce death if the disease is advanced (eight to ten weeks after inoculation); if less advanced (four to five weeks after inoculation) a larger dose, 0·2 to 0·3 gram, is required; but 0·5 gram always proves fatal. The post-mortem appearances are congestion of the lymphatics and viscera, and dark red spots, from mere points to the size of a hemp seed, on the liver and spleen. These are due to enormous dilatation of the capillaries in the immediate

neighbourhood of tubercular deposits, actual extravasations of blood being rarely found. The hæmorrhagic-like spots on the liver are almost pathognomonic of death from tuberculin. Koch found that if tuberculin be mixed with five times its volume of absolute alcohol a brown resinous mass is deposited, the active principle, however, being still in solution. With a larger amount of alcohol the active principle is precipitated with the resinous mass. Ordinary tuberculin contains about 10 per cent. of solid.

If the alcohol be added to the tuberculin in the proportion of 2 : 3, a white flocculent powder is precipitated, which can be purified by washing with 60 per cent. alcohol and finally with absolute alcohol and drying *in vacuo*. This powder seems to contain the active principle of tuberculin. It is soluble in water, but the solution diminishes in activity with age. A solution in 50 per cent. glycerin keeps well for several months and can be exposed to very high temperatures (130° to 160° C.) without diminution of activity. The pure tuberculin gives all the proteid reactions. Picric acid causes a precipitate which dissolves on heating and reappears on cooling; nitric acid also gives a precipitate, but hydrochloric and sulphuric acids do not. It contains sulphur, and from its reactions and elementary analysis evidently belongs to the proteid group, and is most nearly allied to the proteoses.¹

Hunter² carefully examined Koch's tuberculin with the following result. Serum albumin and globulin are absent. It gives the proteose reaction with nitric and picric acids, and also the biuret reaction. Ammonium sulphate completely precipitates the proteid matter, therefore peptones are absent. The proteoses present are proto- and deuterio-albumose, with some hetero-albumose and occasionally a trace of dys-albumose. It also contains traces of mucin, glycerin, extractives, and colouring matter, salts and two alkaloidal bodies. Hunter concluded that the proteoses are the sub-

¹ *Lancet*, 1891, ii. p. 976.

² *Brit. Med. Journ.* 1891, ii. p. 169.

stances possessing remedial and inflammatory actions, while the alkaloidal bodies are fever-producing and not essential to its remedial properties.

Crookshank and Herroun¹ obtained from glycerin broth cultures of the tubercle bacillus a proteose and an alkaloidal body. The proteose was also obtained from 'perlsucht.' Both the ptomine and the proteose (from both sources) produced a rise of temperature in tuberculous guinea-pigs, while in healthy animals the former caused a slight, and the latter a marked, fall in temperature.

De Schweinitz and Dorset² describe some chemical products they have isolated from the tubercle bacillus grown in a special glycerin-asparagin mixture. From the bacilli themselves they have isolated an acid body, probably teraconic acid, an unsaturated acid of the fatty series. A certain amount of the same body was also obtained from the special culture medium, but only a trace from glycerin broth in which the bacilli had been cultivated; in the latter case not because it was not formed, but because of the difficulty of isolation. This acid seemed to produce on injection depression of temperature and necrosis of the tissues locally. It has also some immunising power, and may be the substance in the body producing caseation. The bacilli extracted with hot water yielded an albuminoid, which gave the tuberculin reaction. This they regard as the fever-producing substance.

Koch states that by repeated injections a state of complete immunity towards tuberculin is induced, which may last several months; the immunity, however, is restricted to the toxins and does not exist against the bacilli. The reaction to tuberculin is often lost before a complete cure can be effected, hence the frequent recurrence of the disease, which may again be treated with tuberculin as soon as the patient

¹ *Brit. Med. Journ.* 1891, i. p. 401.

² *Med. Journ. N.Y.* 1897, July 24, p. 105.

again reacts towards it. In endeavouring to obtain substances having an immunising action against the tubercle bacilli, Koch has devised three new modifications of tuberculin.¹ The first, termed T.A. (tuberculin, alkaline), is prepared by stirring and shaking tubercle bacilli with a 10 per cent. solution of caustic soda and filtering. This preparation produces much the same reaction and effects as the original tuberculin, but relapses seem less frequent. It has, however, an insuperable drawback—it produces abscesses at the seat of inoculation when injected in any quantity. The two other new modifications, termed TO. and TR., are prepared by thoroughly triturating tubercle bacilli, emulsifying with distilled water, and centrifugalising. After centrifugalising the emulsion is found to consist of two layers—an upper one consisting of a white opalescent yet transparent liquid free from bacilli, and the lower layer consisting of a muddy residue. This residue is dried, again triturated, and centrifugalised as before, and again yields two similar layers. The same operation is repeated several times until no residue is left. These preparations never give rise to abscesses, and the upper layer after the first centrifugalising has alone to be distinguished from the subsequent ones, the liquids resulting from the second and succeeding centrifugalisations being all alike in their action. The former is the tuberculin O or TO. (O=obera, upper), the latter tuberculin R or TR. (R=residual).

For preparing these new tuberculins it is essential to make use of young and highly virulent cultures of the tubercle bacillus, which must be dried *in vacuo*, and the trituration can only be safely done by machinery.

Tuberculin O closely resembles tuberculin A, but does not cause suppuration; its immunising properties are, however, feeble. Tuberculin R, on the contrary, possesses powerful

¹ *Deutsch. Med. Wochenschr.* 1897, April 1, (translations or abstracts in most of the medical journals).

immunising properties and causes neither reaction nor sup-puration. Healthy guinea-pigs treated with increasing doses of TR. become so completely immunised that they are unaffected by subsequent inoculation with virulent bacilli. This is certainly a most striking result with such a susceptible animal as a guinea-pig. In guinea-pigs first inoculated with virulent bacilli and then treated by injections of TR. retro-gressive changes in the infected organs are always met with, and provided the treatment be commenced within one to two weeks after inoculation, a cure is wrought. The use of tuberculin in treatment will be found in the Appendix.

Tubercle bacilli, living or dead, are with great difficulty absorbed when in any quantity. The dead bacilli when injected under the skin invariably cause suppuration, and several months later it is still possible to detect in the pus numerous bacilli which stain well; introduced into the circulation of rabbits they give rise to nodules in the lungs similar to the tubercular nodules produced by living bacilli (Koch).

The staining peculiarities of the tubercle bacillus are of great importance, for on them is based its detection and recognition in the sputum and tissues. It is difficult to stain, and when once stained retains the dye with considerable tenacity. It stains indifferently with aqueous solutions of the anilin dyes, and it is necessary to use an anilin-water solution of fuchsin or gentian violet, or, better still, the Ziehl-Neelsen carbol-fuchsin solution, and warming the solutions facilitates the penetration of the dye. When stained in this way it can be washed in strong aqueous solutions of the mineral acids (33 per cent. nitric or 25 per cent. sulphuric acid) without becoming decolourised, a peculiarity possessed by only two other bacteria, the smegma bacillus and the *bacillus lepre*.

Other organisms are decolourised by this process, as are also the tissues, sputum, &c. Koch states that this peculiar

staining reaction of the tubercle bacillus is due to a coating of two fatty acids, which take up the stain and are not decolourised by the mineral acid.

The thermal death-point of the bacillus is not high. Sternberg found that tubercular sputum exposed for ten minutes to a temperature of 90°, 80° and 66° C. failed to infect guinea-pigs on inoculation, while another specimen of the same sputum heated for ten minutes to a temperature of 50° C. produced tuberculosis in a guinea-pig, so that from these experiments the thermal death-point lies between 50° and 66° C.

Yersin in 1888, by culture methods, failed to obtain any growth from bacilli which had been heated to 70° C. for ten minutes, while those heated to 55° C. and 60° C. gave growths in glycerin broth in ten days and twenty-two days respectively. Macfadyen and the writer, in the course of some experiments on the sterilisation of milk, found that milk to which powdered dried sputum had been added was rendered innocuous by a momentary heating to 67°-68° C. These experiments indicate that a temperature of 65° C. and over is probably rapidly fatal to the tubercle bacillus, so that milk which has been pasteurised (i.e. heated to 68°-70° C. for twenty to thirty minutes) may be regarded as quite safe. Experiments by the Royal Commission on Tuberculosis with virulent tubercle milk gave somewhat irregular results; in one instance heating to 65° C. for two and a half minutes rendered the milk innocuous, in another instance after five minutes at 70° C. it was slightly virulent, but twelve minutes at the same temperature rendered it inert.

The tubercle bacillus has considerable resistance to the action of antiseptics and germicides. Yersin found that it was killed by 5 per cent. carbolic acid in thirty seconds, by 1 per cent. in one minute, by absolute alcohol in five minutes, mercuric chloride, 1-1000, in ten minutes. Crookshank found that tubercular sputum mixed with an equal volume of 5

per cent. carbolic was rendered innocuous in a few minutes, and this without any special precautions as to breaking up the masses. For disinfecting sputum mercuric chloride is unsuitable.

The distribution of the bacillus in the tissues varies considerably. In young and active tubercles the bacillus is more numerous and more easily demonstrated than in older and more chronic ones. It tends to be more numerous in some animals than in others—in the ox and horse than

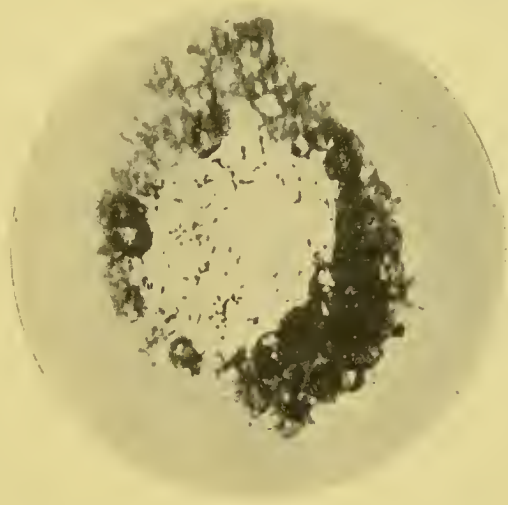


FIG. 41.—GIANT CELL CONTAINING TUBERCLE BACILLI. $\times 750$

in man, for example. In man the bacillus is very difficult to demonstrate (by staining) in enlarged and caseating glands, in pus, in synovial membranes, and in lupus. In some animals, especially the ox and horse, the bacilli can usually be readily demonstrated and may be present in large numbers, and frequently they have the typical distribution, viz. within and at the periphery of the giant cells, though they are by no means confined to this locality (fig. 41). Most, if not all, of the domestic animals are subject to tuberculosis.

It is most common in the ox, pig, and horse, and much less so in the sheep and goat, cat and dog. Wild animals in a state of captivity are also specially prone to be attacked, and a large number of the deaths at the Zoological Gardens, London, are due to this disease, which is also very frequent among birds in captivity. All authorities are agreed that the bacilli of human and bovine, and probably of mammalian tubercle generally, are identical, although their numbers and distribution, and the characters of the lesions they produce, vary in different animals.

Bird or avian tubercle, however, differs in many respects from mammalian tubercle. The tubercular new formations may be very large, but do not show nearly such a disposition to caseation or suppuration as in human tuberculosis. The epithelioid cells form the major part of the growth, and giant cells are very infrequent. One remarkable feature is the enormous numbers of bacilli which may be present in the tissues; in places they may be so numerous and closely packed as to form distinct masses or nodules. The bacilli in avian have the same staining reaction as those of mammalian tubercle, but on cultivation and inoculation various differences between the two forms become evident.

The mammalian bacilli, as before stated, flourish best at about 37° C. and growth ceases at 41° C., whereas the avian bacilli thrive luxuriantly at 43° C., and the growth on glycerin agar is much moister and more wrinkled, and often more pigmented than that of the former. Fowls and dogs are with difficulty infected with human tubercle, but dogs are susceptible to infection with avian tubercle. In all probability the avian bacillus is a variety of the mammalian and not a distinct species, especially as Fischel, by cultivation on boric acid agar and on eggs, has transformed the cultural characters of mammalian into the avian. The subject of avian tubercle is of some practical importance, as several cases are now recorded in which the bacilli cultivated from

human cases were of the avian type, and probably derived from an avian source of infection.

Nocard describes two types of tuberculosis in the horse, one in which the tubercular lesions are chiefly abdominal, while in the other the lungs and bronchial glands are most severely affected. From a long series of observations he has come to the conclusion that, generally speaking, the bacillus obtained from the pulmonary variety is of the ordinary mammalian type, while that of the abdominal one belongs to the avian.¹

The occurrence of tuberculosis in the domestic animals raises some points of practical value, especially the occurrence of infection from the consumption of meat and milk from diseased animals. It has been repeatedly demonstrated experimentally that the ingestion of food containing tubercle bacilli will set up tuberculosis. In the ox the tubercular lesions are most frequently met with in the lymphatic glands and serous membranes, especially the pleura, and in the lungs and liver, while the fat and muscular tissues, which constitute the major part of 'meat,' are very rarely affected. On the pleura the growths take the form of nodular masses, which from their arrangement are popularly termed 'grapes' or 'angle berries.' There can be no doubt that the carcase of an animal extensively affected with tubercle, especially if wasting has occurred, should be condemned as unfit for food, and likewise all parts in which there are tubercular deposits. But it becomes an important question for the community, financially as well as from a hygienic point of view, as to the method of procedure with the meat from a beast comparatively slightly affected with tubercle—an enlarged gland or two, and a few nodules on the pleura. No doubt the ideal method is the condemnation and destruction of all tuberculous carcasses, be the amount of tubercle ever so little; but from a financial point of view this becomes almost an impossibility because of the large amount

¹ *Journ. of Comp. Path. and Therapeut.* ix. 1896, p. 173.

which would have to be paid as compensation. Experiment has demonstrated that the tubercle bacilli are practically confined to the tubercular areas and are extremely rarely met with in the muscular tissues, and these portions, therefore, it might seem, can be consumed with impunity, especially as they would be cooked before consumption.

The Report of the Royal Commission on Tuberculosis, however, indicates two dangers. Firstly, in cutting up a carcass the butcher will most likely use the same knife throughout, and in this way he may infect the meat with tuberculous matter by smearing with the knife. Secondly, cooking cannot be depended upon to destroy the bacilli unless the joints are under 6 lb. in weight; when the weight is above this the temperature in the interior may not rise sufficiently high. Evidently one of the first things to be done is the abolition of private slaughter-houses and the establishment of municipal abattoirs where the meat has to be passed by competent inspectors. In this way all badly affected carcasses would be condemned, and those only slightly affected could be separately dealt with and special precautions taken to eliminate tubercular pieces, &c.

Tubercular milk also raises many important points. It has been suggested that the frequency of intestinal and abdominal tuberculosis in young children is connected with the consumption of tubercular milk, the incidence of these diseases falling just when cow's milk is the staple article of their diet. It is generally acknowledged now that tubercle bacilli do not find their way into milk, even from an extensively diseased cow, unless the udder is affected. When this is the case the bacilli may be met with more or less numerously, and are apparently extremely virulent, more so than is usual. As guinea-pigs and rabbits fed on tubercular milk readily become infected there must be, to say the least, considerable risk to young children who might consume such milk. To avoid risk, all milk intended for the food of

infants and young children should be pasteurised by heating to a temperature of 68° to 70° C. for twenty minutes, or sterilised by boiling.

Much might be done by the registration of all dairy premises and the elimination of all tubercular animals from dairies, and by enforcing the inspection of dairy cattle by competent inspectors at intervals of not more than a fortnight, making the notification of any disease of the udder compulsory, and the sale of milk from such a diseased udder illegal under a heavy penalty (Roy. Com. Tub.). The matter, however, presents considerable difficulty. The ideal method, and one which commends itself at first sight as being the most satisfactory, is the slaughter of all animals which are tuberculous. This was adopted in the State of Massachusetts; under an order of the Board of Cattle Commissioners all beasts in the State were tested with tuberculin, and every animal which reacted was slaughtered, and strict quarantine combined with the tuberculin test imposed on all imported cattle. Even in this small State such a plan was found to be unworkable, the expense being so heavy; and in Great Britain for such a system several millions would probably be required to settle the claims for compensation. A middle course will have to be adopted—all manifestly tubercular animals, especially where wasting or tubercular udder is present, to be slaughtered; animals to be tested with tuberculin, and those which react to be separated from the healthy and to be disposed of as soon as convenient, and in the meanwhile kept as much as possible in pasture.

Every available method should be adopted by local authorities and others to prevent the spread of tuberculosis. It can hardly be doubted that the disease, or at least phthisis, should be made notifiable, though there are many difficulties in carrying it out. Patients should be warned of the danger of disseminating their expectoration, and should use spittoons containing an antiseptic, and handkerchiefs (such as the

paper ones) which can be destroyed. Rooms which have been inhabited by tuberculous patients should be disinfected, for which purpose Délepine recommends spraying with 1-100 solution of chloride of lime. Although cases of direct infection can be rarely proved, the possibility of its taking place cannot be ignored.

Anti-tuberculous sera have been prepared by Paquin in America and Maragliano in Italy, the latter by the injection of animals with tuberculin, but it cannot be said that any very striking results have been obtained by their use.

CLINICAL EXAMINATION.

The examination of sputum, &c. for the tubercle bacillus is now a routine procedure and is often of the greatest value in forming a diagnosis. Fortunately, owing to the peculiar staining reaction of the tubercle bacillus discovered by Koch, the method is comparatively simple.

I. Sputum.—Cover-glass specimens are prepared by smearing a little of the sputum over a cover-glass so as to form a thin film by means of a platinum needle, or by placing a particle of the sputum on a cover-glass, applying another cover-glass, pressing together, and then drawing apart so that a thin film is left on each cover-glass. The thick portion of the sputum should be used, the thin mucoid portion being rejected. If there are any small yellow caseous particles present, these should be chosen, and sufficient material should be placed on the cover-glass to form a distinct but not too thick film; a little experience will soon decide the right amount. Preparations may also be made by smearing the sputum on a clean slide or between two slides instead of using cover-glasses. Whichever plan is adopted, the film is dried and fixed in the usual manner and then stained by one of the following methods :—

(a) Warm carbol-fuchsin two to five minutes (one minute is often quite sufficient). The stain is warmed in a watch-glass or on the cover-glass or slide, holding with a forceps over a Bunsen or spirit lamp until it steams. It should not be allowed to boil, and on the cover-glass or slide fresh stain should be added as

evaporation takes place. After staining, the preparations are washed in water and then decolourised by treating with 25 per cent. sulphuric acid or 30 per cent. nitric acid. In decolourising, when the preparation is immersed in acid in a watch-glass or small breaker its colour changes to a yellowish brown, and on rinsing in water a good deal of the pink colour returns. The treatment with acid and water alternately is repeated until the preparation is practically colourless when rinsed in water. With sputum this is usually the case after three or four rinses in the acid, but it varies with the thickness of the film and with the number of tubercle bacilli present; when these are absent the film usually decolourises much more readily than when many are present. The presence of blood renders the decolourisation difficult. After decolourising and washing, the preparations are stained for one minute in Löffler's methylene blue, washed in water, and mounted in water, or, better, dried and mounted in Canada-balsam or cedar-oil. When the preparation is made on the slide, after washing and drying, it can be examined directly without a cover-glass with the oil immersion after applying a drop of cedar-oil.

The tubercle bacilli appear as delicate red rods, often beaded or segmented, on a blue background composed of cells, mucus, and putrefactive or other bacilli. Occasionally here and there a little red colour may be present in addition to the tubercle bacilli. Hair and keratinised material generally, such as horny epithelium, retain the red colour after the foregoing treatment, and the spores of bacteria are also liable to retain the red longer than anything else, with the exception of the tubercle bacilli. These exceptions are not, however, likely to prove a source of error, for the tubercle bacilli should be recognised not only by their red colour, but also by their characteristic size, shape, and general appearance. For the microscopical examination, a $\frac{1}{6}$ in. with good illumination is sufficient when the tubercle bacilli are present in any number. When they are scanty it is necessary to use a $\frac{1}{12}$ in. oil immersion, and this is the better lens in any case.

If the tubercle bacilli are not found, other specimens should be prepared and examined. *It is only by repeated examinations at different times that the negative evidence of the absence of tubercle bacilli becomes of any value.*

Various methods have been recommended for solution of the sputum and the examination of the sediment for the bacilli after sedimentation or centrifugalising, but they are more or less complicated and do not present any advantage if several specimens are examined.

If it is inconvenient to examine the sputum for a day or two a little 1-20 carbolic should be added. This preserves the sputum, and the tubercle bacilli seem to retain their staining power unimpaired in such a mixture, certainly for many months.

If the tubercle bacilli cannot be detected microscopically after repeated examinations, and it is important to make a diagnosis, the inoculation method may be employed. A couple of guinea-pigs may be inoculated subcutaneously in the thigh or abdomen with 0.5 to 1 c.c. of the sputum. If tubercle bacilli are present the animals will show signs of tuberculosis in three to six weeks (see below, *Urine*).

(b) *Gabbet's Method*.—Prepare and stain the cover-glass specimens in carbol-fuchsin as in method (a). Then wash and treat with the following solution for two to three minutes; wash, dry, and mount :—

Alcohol	50 parts
Water	30 parts
Nitric acid	20 parts

Saturate with methylene blue and filter.

This solution decolourises and at the same time counter-stains.

(c) *Gibbes' Method*.

A. Rosanilin hydrochloride	3 grams
(Fuchsin will do)	
Methylene blue	1 gram
Rub together in a mortar.	
B. Anilin oil	5 c.c.
Rectified spirit	20 c.c.

Mix, and add drop by drop to A, stirring vigorously.

Add 20 c.c. distilled water and preserve in a stoppered bottle.

For use, warm the solution and stain for four to five minutes; wash in spirit until all colour ceases to come out; rinse in water, dry, and mount.

This method has been little employed of late years, but in the writer's hands has given good results.

II. Tissues.—The histological appearances of tubercle are usually sufficient for diagnostic purposes without the necessity of demonstrating the tubercle bacilli, which in many instances may be a very difficult and tedious matter. Sections should be prepared by either the freezing or paraffin method, and stained with hæmatoxylin and eosin, or orange-rubin, or with the Ehrlich-Biondi mixture.

In order to demonstrate the tubercle bacilli the sections are stained in warm carbol-fuchsin for about ten minutes. The stain may be contained in a watch-glass or small glass capsule, and is warmed until it steams, but not boiled, on a wire-gauze tripod or a sand bath. After staining the sections are washed in water and are then decolourised in 25 per cent. sulphuric acid. This takes some minutes, and the sections after being in the acid for a few seconds are washed in water and then returned to the acid, and this alternate rinsing in acid and in water is repeated until they are colourless or nearly so when placed in water. It is not necessary to remove the colour absolutely; a faint pink remaining does no harm. After rinsing in fresh water to remove all the acid, the sections are counter-stained in Löffler's methylene blue for two minutes, rinsed in methylated spirit, passed pretty rapidly through absolute alcohol to avoid removing too much of the blue, cleared in cedar-oil or xylol, and mounted in balsam. The sections may also be counter-stained with hæmatoxylin or Bismarck brown.

Instead of using the strong acid solution for decolourising an acid alcohol solution may be used with advantage.

Gram's method may also be used, but is, of course, not distinctive for the tubercle bacillus.

The following is an improved method for staining tubercle in sections :—

1. Fix tissues by means of perchloride of mercury, acidulated or not, and then harden in alcohol as usual.
2. Embed tissues in paraffin, using toluol as a solvent.
3. Fix sections on slides by means of glycerin albumen in the usual way.

So far there is nothing new in the method.

4. Stain with hæmatin solution for ten to twenty seconds to obtain a pure nuclear stain (not too deep), then wash thoroughly in water.

5. Stain now with Ziehl's carbolised fuchsin, kept at a temperature of about 47° C. for twenty to thirty minutes. The slides are during that time kept in a moist chamber to prevent the stain drying on the specimen.

6. Remove the stain, and treat the section with 2 per cent. watery solution of hydrochlorate of aniline for a few seconds.

7. Decolourise in 75 per cent. alcohol till the section is apparently free from stain; this will take from fifteen to thirty minutes.

8. Double stain with a solution of orange (one part of saturated watery solution of orange to twenty to forty parts of 50 per cent. alcohol).

9. Dehydrate with absolute alcohol.

10. Clear very rapidly with xylol.

11. Mount in xylol Canada-balsam.¹

Where a positive diagnosis is important, a small piece of the tissue may be inserted under the skin of the thigh or abdomen of a guinea-pig. If tubercular, the animal will show signs of tuberculosis in two to three weeks (see below, Urine).

Cover-glass specimens of pure cultivations of the tubercle bacillus may be stained in warm carbol-fuchsin for two to five minutes, rinsed in the sulphuric or nitric acid solution, washed, dried and mounted. They can also be stained by Gram's method, which usually brings out the beaded appearance very markedly. The distinction from the leprosy bacillus will be found at page 214.

III. Urine.—The tubercle bacillus is often very difficult to demonstrate in urine. The urine must be allowed to stand in a conical glass for twenty-four hours, or centrifugalised, and cover-glass specimens prepared with the sediment and treated by one of the methods for sputum given above. Several specimens should be made and must be very carefully examined. If a diagnosis is important inoculation should be resorted to. Two guinea-pigs are inoculated subcutaneously in the thigh or abdomen with 0·5 to 1 c.c. of the sediment from the urine. If tubercle bacilli are pre-

¹ DELEPINE, *Med. Chronicle*, 1896, p. 17.

sent the animals may show signs of tuberculosis as early as two to three weeks after inoculation. Delépine¹ recommends the inoculations to be made on the inner aspect of the leg about the level of the knee. The order of infection after inoculation is as follows: the popliteal, superficial and deep inguinal, and sub-lumbar glands, the retrohepatic, mediastinal and bronchial, deep cervical, and subscapular glands, the spleen, liver, and lungs. The inoculated animals are killed in two to three weeks, dissected, and the lesions examined microscopically. The negative results obtained are nearly as valuable as the positive.

N.B.—Care must be taken not to mistake the smegma bacillus for the tubercle bacillus in urine. For its distinction see page 215.

IV. Milk.—Milk may be treated like urine, allowed to sediment, or centrifugalised, and cover-glass specimens of the sediment prepared, stained, and examined. The only certain method, however, is to inoculate guinea-pigs (as for urine).

PSEUDO-TUBERCULOSIS.

In a number of conditions, collectively termed 'pseudo-tuberculosis,' lesions are met with simulating those of tuberculosis, and distinguishable only by a careful microscopical examination. Such are due to the action of chemical agents, to parasitic worms, to mould fungi, and to certain bacterial forms.²

A 'bacillus pseudo-tuberculosis' has been described by Pfeiffer. It occurs as a shortish, thickish rod, is sometimes coccoid, and can be readily cultivated. Inoculated into mice, guinea-pigs, and rabbits it produces death after days or weeks with the formation of tubercle-like nodules in the organs. The bacillus does not stain by Gram's method, which forms a distinction from the tubercle bacillus.

¹ *Brit. Med. Journ.* 1893, ii. p. 664. The results only apply to ordinary forms of tuberculosis, and not to certain modified forms such as lupus and the avian variety.

² See *Centr. f. Bak.* xv. 1894, p. 501.

LEPROSY.

Leprosy, the Elephantiasis Græcorum or true Elephantiasis, is a disease which has existed and has been recognised from the earliest times among the Egyptians, Jews, Greeks, and Romans. It was undoubtedly somewhat prevalent in the British Isles from the twelfth to the fifteenth century, as the many leper houses and enactments against lepers testify. At the same time, no doubt a number of other skin diseases, syphilides, psoriasis, lupus, &c. were at this early period of medical diagnosis confounded with it. In the present day leprosy, although extinct in the British Isles, may be said to have a world-wide distribution, for it is met with in Iceland and Scandinavia, Russia and the Mediterranean coasts; in Persia, India, China, Siberia, and Japan; in Africa from north to south, and in the American continent in many districts, and also in the Pacific Islands. Three varieties of leprosy are described—the tuberculated, the anæsthetic, and the mixed.

Throughout ancient and medieval times leprosy was considered a contagious and communicable disease, as witness the stringent regulations in the Mosaic and other laws for the segregation of lepers, and modern research seems to bear this out, additional confirmation being given to it by the presence of a bacillus with very distinct characters in the leprous lesions.

The *bacillus lepræ* was discovered by Hansen in 1879. In form it resembles the tubercle bacillus, but is slightly more slender; it probably does not form spores, though in stained preparations the same irregularity in staining—namely. the occurrence of unstained intervals, the so called ‘beading’—is met with as in the tubercle bacillus, and is assumed by some to be due to the presence of spores. The organism is non-motile, stains readily with the ordinary anilin dyes, and by Gram’s

method, which brings out the beaded appearance very well ; but the most striking staining reaction is its property of retaining the colour when stained with fuchsin, and subsequently treated with a mineral acid, thus closely resembling the tubercle bacillus, and in tissue sections the same method is used to demonstrate it as for the latter organism.

The *bacillus lepræ* is found in enormous numbers, usually crowded together in bundles or masses, in peculiar rounded cells, the leprous cells, in the leprous tubercles in the skin, liver, spleen, and testicles, and in the affected nerves in the anæsthetic form ; it has also been found in the blood, but only in the febrile paroxysms which set in when the disease is approaching a fatal termination.

Curiously enough, although the organism is present in such enormous numbers and is so readily demonstrable, there is considerable doubt whether it has been cultivated on artificial media or successfully inoculated into animals. Bordoni-Uffreduzzi has isolated an organism from the bone-marrow of a leper and cultivated it on blood serum which possessed the same staining reaction as the *bacillus lepræ*, but differed from it slightly morphologically, being a little longer and thicker ; and Campania has described its cultivation anaërobically ; but most attempts have failed.

A certain number of positive results of the inoculation of leprous material into the lower animals have been reported by Ortmann and others, but here, too, most of the attempts have ended in failure ; while the positive results are open to criticism and may be fallacious, for lepers not unfrequently suffer from coincident tuberculosis, and the animals therefore may have been infected with and have died from tuberculosis.

The differentiation of leprosy from tuberculosis, although the bacilli are so similar, does not in the majority of cases present much difficulty. The large number of bacilli present in the skin and in the leprous lesions elsewhere forms a marked distinction from tuberculosis, while the bacilli themselves

stain much more readily, and with watery solutions in a shorter time, than does the *bacillus tuberculosis*.

Some cases of leprosy, both of the nodular and anæsthetic varieties, have been treated with injections of Koch's tuberculin, which has been found to produce a certain amount of reaction followed by some amelioration in their condition.

CLINICAL EXAMINATION.

1. If cutaneous nodules be present, one is pricked and a little fluid squeezed out, and cover-glass specimens prepared and stained as for tubercle. Large numbers of bacilli, having the same staining reaction as the tubercle bacillus and obtained from the cutaneous structures, are diagnostic of leprosy (excluding the smegma bacillus).

2. In the tissues the diagnosis must be based on the presence of the bacilli in large numbers in the so-called leprosy cells.

Tissue sections are stained in the same manner as for tubercle.

3. Leprosy is not inoculable on animals.

N.B.—It must be remembered that lepers not unfrequently suffer from tuberculosis.

4. The differentiation of the leprosy from the tubercle bacillus by staining methods cannot be said to be satisfactory. By staining in a saturated aqueous solution of fuchsin in the cold for five to seven minutes, and subsequent decolourising with acid alcohol (nitric acid 1 part, alcohol 10 parts), it is stated that the leprosy bacillus is stained, the tubercle bacillus not.

THE SMEGMA BACILLUS.

The smegma bacillus is an organism found in the smegma præputii, between the scrotum and thigh, and between the labiæ. It also occurs in the cerumen and occasionally on the skin.

It is a small bacillus resembling the tubercle bacillus in size and appearance and, like the latter, is difficult to stain, and when stained with carbol-fuchsin retains the colour after

treatment with a 25 per cent. mineral acid (fig. 42). It has, therefore, to be distinguished from the tubercle bacillus in certain localities, viz. in urine and about the external genitals. It is non-inoculable on animals and has only recently been cultivated beyond one generation, by Czaplewski,¹ who grew it on serum and glycerin agar, also in broth, but not on potato; it forms minute (0.5–1 mm.) irregular, roundish colonies. It has been suggested that the syphilis bacillus of Lustgarten is

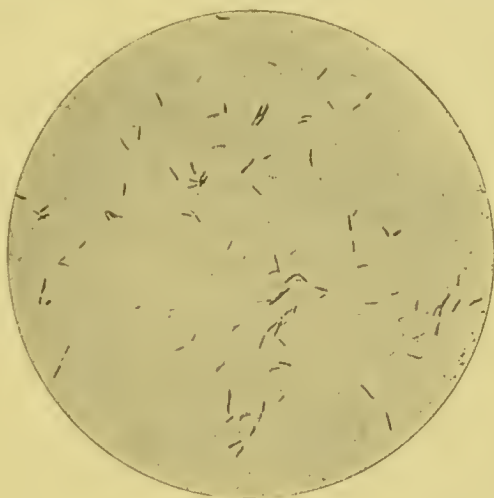


FIG. 42.—THE SMEGMA BACILLUS. NEELSEN'S METHOD. $\times 1000$

identical with the smegma bacillus; they are both decolourised by Lustgarten's permanganate method, but while the smegma bacillus after staining is with difficulty decolourised by acid, and is easily decolourised by alcohol, the reverse is the case with the syphilis bacillus.

STAINING AND DIFFERENTIATION.

Cover-glass specimens of smegma may be stained in exactly the same manner as for tubercle. When staining for tubercle bacilli

¹ *Münch. Med. Woch.* 1897, No. 43.

in urine the urine should be drawn off by means of a catheter ; this will exclude the smegma bacillus.

If there is reason to suspect the presence of the smegma bacillus when staining for tubercle, Bunge and Tranteroth ¹ recommend that the cover-glass specimens should be treated as follows :

1. Immerse in absolute alcohol for three hours.
2. Immerse in 5 per cent. chromic acid for fifteen minutes.
3. Stain in warm carbol-fuchsin.
4. Decolourise in 25 per cent. sulphuric acid for two to three minutes.
5. Counter-stain in a concentrated alcoholic solution of methylene blue for five minutes.

The smegma bacillus will be decolourised by this method.

¹ *Fortschrit. der Med.* xiv. 1896, Nos. 23 and 24. See also *ibid.* No. 9. and Grunbaum, *Lancet* 1897, i.

GLANDERS.

Glanders is a disease known from the earliest times, being recognised by the Greek and Roman writers, by whom it was termed *μάλῆς* and *malleus* respectively. It is especially a disease of the horse, mule, and ass, but is also readily communicable to man, and is not uncommon among those who come in contact with or are in charge of horses.

In the horse the disease especially affects the nasal mucous membrane. Nodules form which afterwards break down and ulcerate, and a muco-purulent discharge appears. In the course of time the disease spreads to the trachea and lungs, in which nodules develop somewhat resembling tubercles. The lungs probably always contain some nodules. The lymphatic glands may also become involved and enlarged, and tumour-like masses may appear under the skin, to which the name of 'farcy buds' is given.

In man the disease occurs in two forms—the acute and the chronic. The former is a very serious affection accompanied by high fever, prostration, and delirium, and almost invariably fatal in from two to three weeks. The seat of infection is usually the hand or arm, the nasal mucous membrane being sometimes subsequently involved, and deposits may form in the lymphatic glands, internal organs and muscles. In the chronic form indolent ulcers are present, the disease runs a prolonged course of weeks or even months, and about half the cases end in recovery. It is stated that in this disease in the early stage an eruption may develop on the forehead and face simulating very closely that of small-pox.

The glanders nodules consist of masses of round cells, with occasionally giant cells. In the horse the nodules in the lungs have a shotty feel; at the centre they consist

of a soft purulent material, around which is a zone of catarrhal exudation, and outside this is another zone of fibrinous or croupous exudation into the alveoli. If the animal lives long enough the nodules may become surrounded with a fibrous capsule, and giant cells may be met with at all stages.

Glanders is due to a small bacillus, the *bacillus mallei*, discovered by Löffler and Schutz in 1882; it is a little shorter and thicker than the tubercle bacillus, is generally solitary,

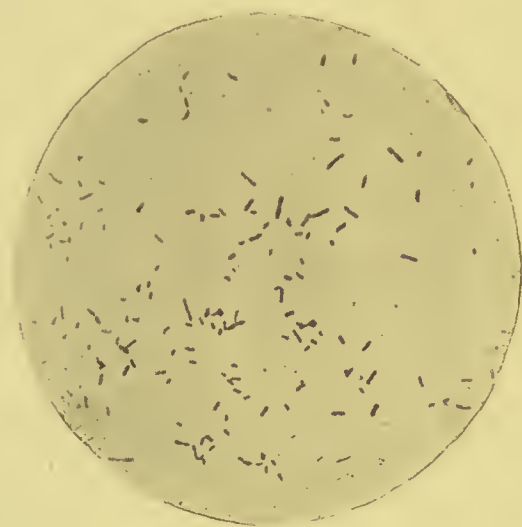


FIG. 43.—GLANDERS BACILLUS. COVER-GLASS PREPARATION. $\times 1000$.

and in stained preparations (fig. 43) often appears more or less beaded. It does not form spores and is probably non-motile, though in a hanging-drop cultivation made from a pure culture an extraordinarily active Brownian movement is observed. The bacilli from a young culture not more than twenty-four hours old readily stain with the ordinary anilin dyes, but after two or three days' growth the bacilli become degenerate and stain badly. In the discharges and tissues, on the other hand, it stains indifferently—in the discharges being most

difficult to demonstrate, and tissue sections require careful staining and examination before it can be found. In the tissues, the staining can be carried out with carbol-methylene blue or carbol-fuchsin as detailed in the section on 'Clinical Examination' (p. 221), or by Unna's method.

The *bacillus mallei* is an aërobic and facultative anaërobic organism. It grows but slightly on gelatin at 22° C., what growth there is being brownish and without liquefaction; on glycerin-agar it forms a thick cream or slightly brown coloured growth; on blood serum a somewhat amber-coloured growth in the form of droplets; on potato at 37° C. in two or three days amber-coloured transparent droplets appear, which subsequently spread, coalesce, and may become deep brown; in broth it produces a general turbidity with a sticky deposit and sometimes a slight film formation; neutral litmus sugar-agar becomes red from the development of acid, and broth cultures give the indol reaction. Milk is not coagulated. The glanders bacillus varies considerably in virulence, and under continued cultivation may become almost non-pathogenic.

Mallein, an analogous preparation to tuberculin, is prepared by growing a virulent glanders bacillus in glycerin veal-broth in flat flasks such as are employed for tuberculin (fig. 40), so that there is free access of oxygen, for a month or six weeks. It is then autoclaved for fifteen minutes at 115° C., filtered through a Berkefeld filter, concentrated to one-fourth of its volume, and mixed with an equal volume of a $\frac{1}{2}$ per cent. solution of carbolic acid. This yields an active mallein, 1 c.c. of which is a dose, and gives a good reaction. Like tuberculin, it has little or no curative properties, though a few cases of cure by prolonged use have been reported by Babes and others. Its main use is for diagnostic purposes, and veterinary authorities are unanimously agreed that it is one of the most certain means of diagnosing glanders in the horse that we possess. Injected into an unglandered horse little or no effect is produced, but

in a glandered animal, about twelve hours after injection, the temperature rises 1.5° to 3° C. above the normal, a large and painful swelling forms at the seat of inoculation (it may be as large or even larger than half a cocoanut), while any affected lymphatic glands or farcy buds become swollen.

Glanders is met with chiefly among horses, asses, and mules, and may be communicated to man by these animals.

Guinea-pigs and field-mice are highly susceptible to the disease, which may also be contracted by some of the Carnivora, as the cat, lion, and tiger, by inoculation or by feeding on diseased carcasses. The rabbit, sheep, and dog are but slightly susceptible, while cattle, swine, and house-mice are immune.

The lesions set up in an inoculated guinea-pig are characteristic and can be used for diagnostic purposes. With a very virulent culture, such as can be obtained by several passages through a susceptible animal, a guinea-pig may die in four or five days, and the post-mortem lesions are slight, consisting of some caseation at the seat of inoculation and slightly enlarged spleen, which contains a few small yellowish nodules resembling miliary tubercles.

More frequently the culture or material from a glandered horse does not produce death until a lapse of two or three weeks. A male guinea-pig being chosen, the changes observed are caseation followed by ulceration at the seat of inoculation, when this is done subcutaneously, and great enlargement of the testicles; on cutting into these they are found to be partially or almost entirely converted into a pasty caseous material, while the skin covering them is so adherent that it can only be detached by cutting, and the spleen is very much enlarged and studded with small yellowish nodules. In a female guinea-pig the ovaries are attacked. These appearances are of importance in the diagnosis of the disease. The difficulty of finding the bacillus in the discharges by microscopical and staining methods is so great that it can hardly be employed with any cer-

tainty. Löffler and Strauss therefore recommend the inoculation of a male guinea-pig intraperitoneally with the discharge or other material. If the glanders bacillus is present the lesions just described rapidly ensue, and the diagnosis is established in four or five days. At the present time the inoculation method has been almost entirely superseded by the introduction of mallein, the former being reserved for clinical diagnosis in man.

MacFadyean¹ has found that the blood of a glandered animal produces agglutination or clumping of the glanders bacilli from a culture similar to that obtained in the Widal test for typhoid, and has suggested this reaction as a means of diagnosis. As an aid to the clinical diagnosis of the disease in man it is doubtful if the method of serum diagnosis can be applied, for Foulerton² has found that typhoid and diphtheria sera also produce agglutination of the glanders bacillus.

The greatest care should be exercised when working with glanders material or cultures, several fatal laboratory accidents having, unfortunately, happened to investigators.

CLINICAL EXAMINATION.

1. Prepare and stain cover-glass preparations of the pus or discharge in the usual manner. The ordinary pyogenic cocci will not be found unless a secondary infection has occurred, and the material will probably appear sterile, for the glanders bacilli are rarely observed.

2. If the material appears nearly sterile, several tubes of glycerin-agar and potato may be inoculated and incubated at 37° C. for twenty-four to seventy-two hours. On the agar, colonies of the glanders bacillus will develop in twenty-four to thirty-six hours, but the potato will not show the characteristic amber growth under forty-eight to seventy-two hours.

3. It will usually be necessary (in man, at any rate) to confirm

¹ *Journ. of Comp. Path. and Therapeut.* 1896, December.

² *Lancet*, 1897, i. p. 1201.

the diagnosis by an inoculation experiment. A male guinea-pig is chosen and a little of the discharge, or an emulsion (0·5 to 1 c.c.), is injected intraperitoneally. In three to five days the animal should show the characteristic swelling of the testicles if the material be glandered.

4. In animals the mallein test may be applied (see Appendix).

5. In animals the Widal reaction may be applied, i.e. testing the effect of a 1:10 or 1:20 solution of the blood serum on the glanders bacillus (see Typhoid, p. 235). In man this test might give an inconclusive result (see *ante*).

6. In the tissues the glanders bacillus is difficult to demonstrate. Sections may be stained for half an hour with carbol-methylene blue, rinsed in 1 per cent. acetic, rapidly dehydrated with alcohol, cleared and mounted, or, to avoid removing too much colour with the alcohol, dehydration may be performed with anilin as in Weigert's method. Abbott recommends¹ the following method : Rinse the sections in distilled water, stain on the slide with a dilute carbol-fuchsin (1:10 water) for half an hour. Wash for ten seconds three times with 0·3 per cent. acetic, wash with distilled water, blot, dry by very gentle heating, clear in xylol, and mount.

¹ *Principles of Bacteriology*, 3rd ed.

CHAPTER X.

TYPHOID FEVER—BACILLUS COLI COMMUNIS.

TYPHOID FEVER.

A BACILLUS originally isolated by Eberth in 1880, and more closely studied by Gaffky in 1884, is now generally regarded as the specific organism of enteric fever. The experimental proof, however, that this is the case is difficult to obtain, for none of the lower animals suffer from a disease which is comparable to enteric fever in man.

The Eberth-Gaffky bacillus, or *bacillus typhosus*, is best observed in sections of the spleen, in which it occurs in groups or colonies consisting of short rods with rounded ends measuring about 3μ . It has also been demonstrated in the mesenteric glands and liver, in the swollen Peyer's patches before ulceration, and it occurs in the urine, fæces, and bronchial secretion, and has occasionally been found in the blood.

In order to obtain pure cultivations it is preferable to make use of the spleen. The organ is washed and then cauterised lineally by means of a red-hot iron in order to destroy the saprophytic bacteria on and near the surface. An incision is made with a sterilised knife through this cauterised area, and a little of the splenic pulp is taken with a sterilised platinum needle and inoculated on to agar tubes, and it is well also to prepare a set of agar plates. These are incubated at 37°C . for twenty-four to forty-eight hours,

and the growths which develop are examined and tested by microscopical and culture methods. The following are the characters of the *bacillus typhosus*:—

Morphology.—Bacilli with rounded ends 2 to 3 μ in length and 0.6 μ broad. It is a very pleomorphic organism, and involution forms invariably occur in cultivation as long threads 10 to 20 μ in length and 1 to 5 μ thick (fig. 44). It does not seem to form spores, although refractile granules may occur, and a spore formation has been described by Almquist.

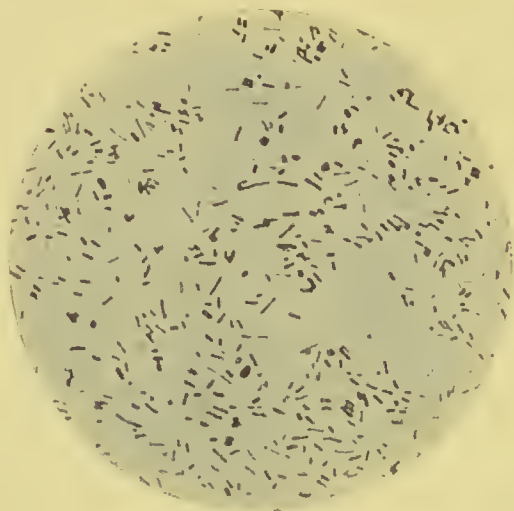


FIG. 44.—*BACILLUS TYPHOSUS*. COVER-GLASS PREPARATION. $\times 1000$.

It is actively motile and possesses a number of flagella, ten to twenty, both at the poles and sides (fig. 45). It stains by the ordinary anilin dyes, but not by Gram's method. In stained preparations unstained spaces, or vacuoles, may frequently be seen.

It is aërobic and facultative anaërobic and grows well on the ordinary culture media. On agar it forms a thick, moist, greyish layer. On gelatin it grows slowly, and the growth, which is scanty, is white and shining and somewhat irregular

(fig. 46). The colonies in gelatin are visible in about forty-eight hours, and form small roundish white points, which are granular and brownish in colour by transmitted light. In broth it produces a general turbidity, without film formation. The growth on potato having an acid reaction is somewhat characteristic; it forms a moist, grey, shining layer which is

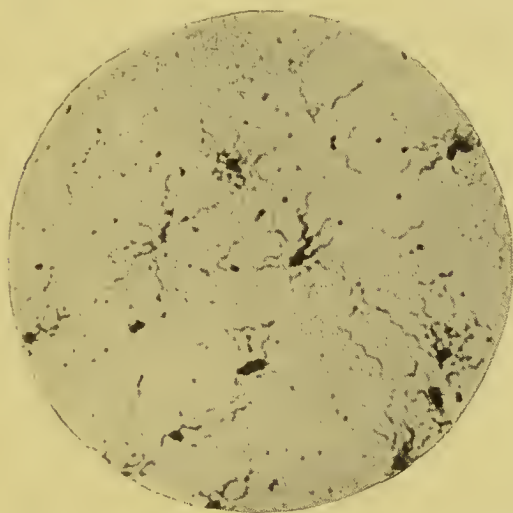


FIG. 45.—*BACILLUS TYPHOSUS*. COVER-GLASS PREPARATION SHOWING FLAGELLA. $\times 1000$. (Van Ermengen's method.)

almost invisible. If, however, the reaction of the potato be neutral or alkaline the growth may be yellowish. It grows well in milk without coagulation.

Acid is formed in small quantity during its growth (volatile fatty acids, and lactic acid), which can be demonstrated by cultivating in litmus milk, or on litmus sugar-agar, and the



FIG. 46.—*BACILLUS TYPHOSUS*. GELATIN SURFACE CULTURE, 7 DAYS OLD.

organism will grow in slightly acid media. Neither gas nor indol¹ is formed in cultures.

The typhoid bacillus is destroyed in ten minutes by a temperature of 56° to 60° C. In cultivations it retains its vitality for months, and when dried for weeks.

From cultures of the typhoid bacillus Brieger isolated a base which he termed typhotoxin and which is isomeric with gadinine. In animals it produced salivation, profuse diarrhoea, paralysis, and death. Brieger and Fränkel have also isolated from cultures a toxic proteid body, while Pfeiffer states that the typhoid poison is contained in the bacterial cells—a view supported by Klein.

Fenwick and Bokenham² have extracted from spleens of enteric fever patients a proteose, an alkaloid, and a fatty residue. The proteose produced fever, anorexia, and loss of weight in guinea-pigs and rabbits, but the alkaloid and fatty matters were without effect.

Injected intraperitoneally into mice and guinea-pigs the *bacillus typhosus* usually produces death, and the same result follows from intravenous injections in rabbits. Given by the mouth, unless the dose be large, no result follows, and the same is the experience of most observers who have fed animals on typhoid stools. A disease process analogous to enteric fever in man has rarely been induced by inoculation experiments, but Rembinger³ states that by feeding young rabbits on vegetables, cabbage, &c. soaked in water to which had been added some culture of the typhoid bacillus, he has succeeded in inducing a condition resembling typhoid fever in man. The charts which accompany the paper show a typical rise of temperature, a period of pyrexia with morning remission, followed by a typical fall of temperature. The animals suffered from diarrhoea, and their blood gave the Widal

¹ Occasionally traces of indol may be detected by careful testing.

² *Brit. Med. Journ.* 1895, i. p. 801.

³ *Ann. de l'Inst. Pasteur*, xi. 1897, p. 829.

reaction. Post-mortem, the intestine was congested and filled with yellow diarrhoeic matter, the Peyer's patches were swollen and in some places commencing to ulcerate. The spleen was increased to two or three times its normal size, and cultures of the typhoid bacillus were obtained from it. The morbid effects following intraperitoneal inoculation are similar to those produced by the *bacillus coli communis* and other species.

The *bacillus typhosus* is found in the sputum and lungs in pneumonia complicating enteric fever; it is also a pyogenic organism, and has been met with in abscesses occurring after enteric fever, and occasionally in osteomyelitis.¹ It is by no means easy to isolate from the stools. Simple plate cultivations usually fail, the best methods being Parietti's and Elsner's (see p. 383).

Block,² by withdrawing a little blood from a vein with a syringe under aseptic precautions and making cultivations on agar, was able to isolate the typhoid bacillus. He states that only three other observers have succeeded in isolating the typhoid bacillus from the blood during life.

The *bacillus typhosus* has been isolated in a few instances from water supplies which have become infected, and have given rise to epidemics. A notable instance of this was the Worthing epidemic in 1892. This is the exception, however, and the isolation of the typhoid bacillus from an infected water is a very difficult matter. In the Worthing water, for example, only two or three colonies were found in 1200 c.c. of water taken directly from the polluted well, and none in double that quantity from the mains, although 300 cases had arisen from drinking it. In the recent Maidstone epidemic also it was not found, although 1800 cases occurred from drinking the polluted Farleigh water. The difficulty is increased from the fact that all the methods devised so far

¹ *Ann. de l'Inst. Pasteur*, x. 1896, p. 220 (C. Bruni).

² *Johns Hopkins Hosp. Bull.* viii. 1897, No. 75, p. 119.

favour the *bacillus coli communis* as much as, or more than, the *bacillus typhosus*; and, in addition, forms have been found in water which, while not actually being the *bacillus typhosus*, resemble it very closely. Such are the so-called pseudo-typhoid bacilli isolated by Cassedebat at Marseilles. Some of the varieties of the colon bacillus likewise closely resemble the typhoid bacillus; these are referred to below (p. 240), and a table of the differences between the typhoid and colon bacilli is given at page 241. The methods of isolation from water are given in Chapter XXI., p. 382.

In sterilised waters, including distilled water, the *bacillus typhosus* maintains its vitality for upwards of a month, and in some cases for much longer. In aërated (CO_2) waters it does not survive a fortnight. In unsterilised water the duration of vitality is uncertain; it usually disappears within a fortnight or three weeks.

In a certain number of cases of enteric fever the evidence has pointed strongly to oysters as a source of infection, and so important was this hypothesis that the Local Government Board investigated the matter. It was shown that in many instances the oyster beds or layings were subject to contamination by sewage, in some cases the sewage from towns being voided at or close to the oyster-beds. From many samples of oysters subject to sewage contamination Klein obtained the colon bacillus in large numbers, and from one oyster a cultivation in phenol broth gave a pure culture of the typhoid bacillus. Further, by inoculating oysters with the typhoid bacillus and keeping them alive in tanks of sea-water, the typhoid bacilli were obtained from their interior four, nine, sixteen, and even eighteen days from the commencement of the experiment, the oysters showing no abnormal condition, while the typhoid bacilli recovered from them and from the tank water retained unimpaired all the characters of a typical typhoid bacillus. The investigations of the Local Government Board Inspectors and the experiments of Dr. Klein

show, therefore, that infection from oysters and other shell fish is quite possible.¹

As regards the vitality of the *bacillus typhosus* in sewage, Parry Laws and Andrews² show, in the first place, that in ordinary sewage the mathematical chances of detecting the bacillus are extremely remote, but that in the sewer from a typhoid block at the Eastern Hospital, Homerton, where the stools had not been disinfected for two days, a bacillus was isolated which was identical with the *B. typhosus*. In sterilised sewage inoculated with it the *B. typhosus* hardly multiplied at all, and at the end of ten days had died out. Certain organisms in sewage seemed to have a deleterious action on the *B. typhosus*, hastening its extinction, viz. the *B. fluorescens liquefaciens* and *B. fluorescens stercoralis*.

In dry garden earth, according to Dempster,³ the *bacillus typhosus* does not live longer than eighteen days, and in peat it dies within twenty-four hours. In moist soil, however, the bacillus was still alive on the forty-second day. In an artificially dried soil it was not found alive after the seventh day.

Cultures of the *B. typhosus* do not give Ehrlich's diazo reaction.⁴

In a research on the conveyance of infection by the air, Germano⁵ has investigated the duration of life of the typhoid bacillus in dust, on clothing, and in fæces. In dust the typhoid bacilli always die quickly on desiccation (within three days), but when kept damp they live much longer. In dust and sand infected with typhoid stools much the same result was obtained. On clothing, however, even when dried, the bacillus

¹ Local Government Board Report, 'On Oyster Culture in relation to Disease.' 1896.

² 'Report on the Micro-organisms of Sewage.' Reports to the London County Council, 1894, No. 216 (Stanford & Co.).

³ Med. Chirurg. Trans. lxxvii. 1894, p. 263.

⁴ Brit. Med. Journ.

⁵ Zeitschr. f. Hyg. xxiv. 1897, No. 3, p. 403.

retained its vitality for more than a month. He considers from these experiments that the conveyance of the typhoid infection through the air, at any rate for any distance, is very unlikely.

There has always been considerable discussion on the exact relation of sewer-gas to disease. In enteric fever, diphtheria, and tonsillitis most physicians hold that the inhalation of sewer-gas is, to say the least, a predisposing cause. Some have considered that the specific organisms are present in the emanations from sewers, and this is possibly the case occasionally. Alessi,¹ however, has, by some interesting experiments, put the relation of sewer-gas to disease on a firmer basis than hitherto. He placed animals, rats, rabbits, and guinea-pigs, in a box with a perforated bottom communicating directly with a drain. After exposure for a varying period the animals were inoculated with a small quantity of a slightly virulent culture of the typhoid bacillus. The rats, after inhaling the foul air, lost their vivacity and became emaciated, and out of forty-nine inoculated with the typhoid bacilli thirty-seven died, while of forty-one control animals similarly inoculated only three died. The result was the same with guinea-pigs and rabbits; of seventy-two guinea-pigs exposed to the sewer-gas and then inoculated fifty-seven died, whilst none of the control animals succumbed; every one of the eleven rabbits similarly treated died, but none of the control animals. It was found that the animals were most susceptible to the typhoid infection during the first two weeks of exposure to the sewer-gas, for no less than 90 per cent. inoculated during this period died, whilst during the third week only 76 per cent. succumbed. The inference from these experiments is that sewer-gas lowers the power of resistance of the body to infection, but that by long exposure a tolerance may be established.

The proof of the causal relation of the *bacillus typhosus*

¹ *Nature*, vol. 1. 1894, p. 19.

to enteric fever is based on the following facts. It is met with in the tissues in cases of enteric fever, and has been obtained from the spleen during life by puncturing with a hollow needle, and this method has been suggested as a means of diagnosis. It has also been isolated from the urine and blood during the course of the disease, and it is not met with in other diseases. Much stronger evidence has recently been adduced by the introduction of what is known as Widal's reaction. The blood and blood serum of an animal

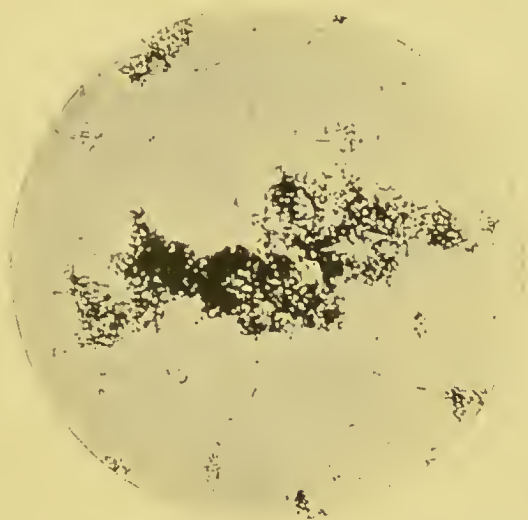


FIG. 47.—WIDAL'S REACTION. A CLUMP OF BACILLI. (Before the addition of the serum the typhoid bacilli were quite isolated.) $\times 350$.

immunised against the *bacillus typhosus* are found to bring about cessation of movement and agglutination of the bacilli in a broth culture of the organism (fig. 47). A similar result occurs when the serum of a patient, in the second week of an attack of enteric fever, acts on the *bacillus typhosus*, the reaction not occurring with healthy individuals or in other diseases. This seems to be strongly confirmative that the *bacillus typhosus* is the specific organism of enteric fever, for

it indicates that in the body of an individual suffering from enteric fever the same substances are formed as in an animal artificially immunised by cultures of the *bacillus typhosus*. This reaction is now being used as a clinical test in doubtful cases of enteric fever.

The following conclusions were arrived at in a discussion on the Widal reaction at the American Congress of Physicians and Surgeons, 1897 :¹

‘ In spite of considerable variation in technique there has been a remarkable uniformity in the results obtained by those taking part in the discussion, and their average of about 95 per cent. of successes agrees with the general average of the cases—nearly 4000—thus far recorded in medical literature. Each of several methods of technique advocated may thus give good results in the hands of those thoroughly familiar with the details found necessary in each case, and the sources of error to be avoided ; success depending rather on being perfectly familiar with one method than on the particular one selected.’

‘ A complete reaction should comprise both characteristic clumping and total arrest of motion occurring within a definite time limit. For practical diagnostic work a dilution of 1 : 10, with a fifteen-minute time limit, is convenient. In any doubtful case the dilution should be carried as far as 1 : 50, or perhaps 1 : 60, and a reaction not obtainable at that point should not be regarded as perfectly conclusive. For these higher dilutions the time limit should be extended to two hours.’

‘ The evidence so far recorded establishes that the reaction may be delayed, or occasionally may not be obtained in cases of genuine typhoid infection ; and also that it may be exceptionally present in non-typhoid cases, though not in an intense degree.’

‘ Without being absolutely infallible, the typhoid reaction

¹ *Brit. Med. Journ.* 1897, ii.

appears to afford as accurate diagnostic results as can be obtained by any of the bacteriological methods at our disposal for the diagnosis of other diseases. It must certainly be regarded as the most constant and reliable sign of typhoid fever, if not an absolute test.'

Johnson and McTaggart¹ state that typhoid blood dried for sixty days still gave a typical 'Widal' reaction. An incomplete reaction was occasionally obtained as early as the end of the second day, and the complete reaction was rarely delayed beyond the fifth day. They also noticed that the blood of the horse often produced clumping, &c. of typhoid bacilli, indistinguishable from a 'Widal' reaction with typhoid blood; but the same agglutinating effect is also produced on the colon bacillus. Many chemical substances also produce agglutination of typhoid bacilli, so that it is necessary to exclude them in making a diagnosis. For example, corrosive sublimate (0.7 : 1000), alcohol, salicylic acid, vesuvium, and safranin (1 : 1000) agglutinate, while carbolic and lactic acids, chloroform, caustic soda and ammonia do not; the last two only, provided the test typhoid emulsion be made with distilled water. Safranin has a powerful agglutinating action on the typhoid bacillus, but not on the colon bacillus.²

Widal and Sigard³ in an exhaustive paper on the serum diagnosis recommend that one drop of serum be mixed with ten drops of a bouillon culture. They conclude that the reaction can be generally observed in typhoid fever from an early period of the disease, and that it is only exceptionally delayed. The phenomenon of agglutination is not a vital reaction on the part of the organisms. A negative result obtained with the serum of a suspected case furnishes a probability against the diagnosis of typhoid fever, but only a

¹ *Brit. Med. Journ.* 1896, ii. p. 629.

² Malvoz, *Ann. de l'Inst. Pasteur*, xi. 1897, p. 582.

³ *Ann. de l'Inst. Pasteur*, xi. 1897, No. 5, p. 353.

probability, especially if the examination has been made during the early stage of the disease; it should then be repeated, and this probability becomes greater if the examination be made at a later stage of the disease. A positive reaction should be considered as a conclusive sign of typhoid fever.

Foerster¹ finds that the agglutinating action of the serum of typhoid cases on the typhoid bacilli is obtainable when it is diluted from sixty to 5000 times. The most active normal serum (met with once in ninety cases) did not produce any agglutination when diluted more than forty times. There is no constant connection between the activity of agglutination and the severity of the disease. In convalescence the serum produced agglutination in the fifth month. Nine different typhoid cultures tested with the same serum did not show much difference in the amount of agglutination, the ratio of the least to the most being 5 : 8.

The bactericidal action of the blood serum did not undergo any constant alteration as a result of the typhoid infection. The agglutinating and bactericidal actions of the blood serum are not due to the same substance, for while the bactericidal action is destroyed by heating to 55° C. for half an hour, the agglutinating action is unaltered by this treatment.

An attempt has been made by Ransome and others to obtain an antitoxin by the inoculation of animals with cultures of the *bacillus typhosus*, but it cannot be said up to the present that the antitoxin treatment of enteric fever has had much success.

CLINICAL DIAGNOSIS.

1. *Puncture of the spleen with a sterilised hypodermic needle and syringe.*—A little of the blood and pulp is withdrawn with the syringe and cultivations made on agar, and the tubes incubated for twenty-four hours. This method seems

¹ *Zeitschr. f. Hyg.* xxiv. 1897, No. 3, p. 500.

hardly justifiable, and now that the Widal reaction has been introduced should be discarded.

2. *Examination of the Stools*.—The only method likely to give a satisfactory result is by the employment of Elsner's potassium-iodide-potato-gelatin medium. Three series of plates of three each should be made, the first tubes in each series being inoculated with two, four, and six loopfuls, respectively, of the stool. The plates are examined on the second and third days (see p. 384).

3. *Widal Reaction*.—The lobe of the ear or the finger having been carefully cleansed, preferably by washing with 1 : 20 carbolic, and then with alcohol and with ether, is pricked with a sterile needle so that two or three drops of blood exude. A steel nib with one half broken off forms a good lancet. The drops of blood are drawn up into a capillary tube having a bulb in the middle (like fig. 6, p. 39) and sealed up, and may then be manipulated at leisure, preferably the next day. If no capillary tubes are at hand the blood may be spotted on to a cover-glass or glass slide and allowed to dry, or on to a piece of note-paper or absorbed with fragments of blotting paper. A recent broth culture of the typhoid bacillus, preferably not more than twenty-four hours old, is employed. An agar culture one, two, or even three days old may also be used, 3 or 4 c.c. of distilled water being poured in, gently shaken, and poured off again into a sterilised tube. In either case the broth culture or emulsion should be filtered before use, for a certain amount of clumping will generally be found in the cultures and is somewhat confusing. The filtering is done in the ordinary way through a paper moistened with distilled water; this removes all but the smallest clumps, while the isolated bacilli pass through. A hanging drop (p. 101) is then made with the filtrate and should show plenty of isolated bacilli, actively motile, and practically no clumps. Having ascertained that the culture is in good condition, a dilution of the blood or serum is prepared. The ends of the pipette are broken off and a drop of the serum or blood is blown out on to a clean cover-glass or slide. A small loopful of this is then placed in the hollow of a hollow slide, and with the same loop five loopfuls of sterile broth, salt solution, or water are added, and the whole mixed up. A loopful

of this solution is then mixed with an equal loopful of the filtered typhoid culture on a clean cover-glass and a hanging drop prepared. It is still simpler and, perhaps, better, to mix one loopful of the serum or blood with ten loopfuls of the filtered typhoid culture and make a hanging drop with this mixture. Care should be taken that the hanging-drop cultures are quite sealed with the vaseline so that evaporation is prevented. The hanging drops are then examined under the microscope, a $\frac{1}{6}$ -in. objective being sufficient for the purpose, and should be observed for half an hour. In a well-marked case the following phenomena will be observed. The motility of the bacilli is instantaneously or very quickly arrested, and in a few minutes the bacilli begin to aggregate together into clumps, and by the end of the half-hour there will be very few isolated bacilli visible. In less marked cases the motility of the bacilli does not cease for some minutes, while in the least marked ones the motility of the bacilli may never be completely arrested, but they are always more or less sluggish as compared with the control hanging drop, while clumping ought to be quite distinct by the end of the half-hour.

When the blood has been dried on glass or paper, a droplet of water must be added and a solution made, which is treated in the same manner as blood or serum.

Dead bacilli can also be made use of, and might prove useful in a foreign country or elsewhere, where there are no facilities for cultivation. They may be prepared by heating a broth culture to 65° C. for ten minutes and then preserving the fluid in sterilised glass pipettes.¹

N.B.—The above description of the serum diagnosis must be regarded as provisional, for papers relating to it are now appearing weekly, and the best conditions for obtaining it have not yet been finally settled.

BACILLUS COLI COMMUNIS.

The *bacillus coli communis*, or, as it is frequently called, the *bacterium coli commune*, or colon bacillus, is an organism

¹ Délepine, *Brit. Med. Journ.* 1897, i. p. 967. (Full. refs.) Wright, *Ibid.* 1897.

of considerable importance, both in connection with the *bacillus typhosus* and in pathological processes. As its name implies, it is a constant inhabitant of the intestinal tract in man and animals; and is, perhaps, one of the most widely distributed organisms in nature.

In order to isolate it all that is necessary is to inoculate two or three Parietti broth tubes (containing 0·2 c.c. of Parietti's solution, see p. 383) with a platinum loopful of fæces, incubate for twenty-four hours at 37° C., and then prepare

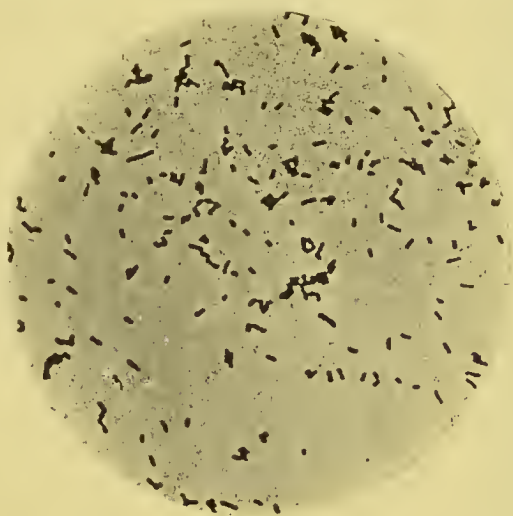


FIG. 48.—THE COLON BACILLUS. COVER-GLASS PREPARATION. $\times 1000$.

gelatin plate cultures. The organism has the following characters :—

It is a short rod with rounded ends, 2 or 3 μ long and 0·4 to 0·6 μ broad, frequently linked in pairs or more. It is often so short that it is merely ovoid in shape; and, on the other hand, longer individuals and involution forms occur 6 μ or more in length (fig. 48). It is feebly motile, and possesses lateral flagella to the number of from 2 to 10.

Spore formation does not seem to occur, although it has

been described by Almquist, but vacuolation may sometimes be observed. It is well stained by the ordinary anilin dyes, but is decolourised by Gram's method.

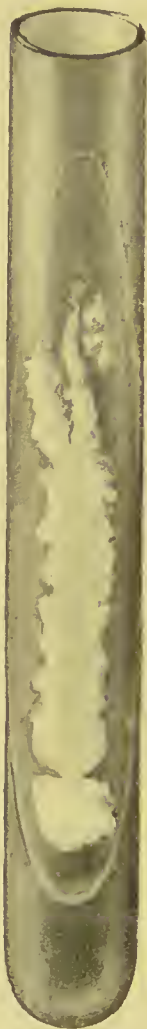


FIG. 49.—COLON
BACILLUS.
SURFACE GELATIN
CULTURE, SEVEN
DAYS OLD.

It is aërobic and facultative anaërobic, and grows readily on the ordinary culture media from 20° C. to 37° C. In gelatin plates the colonies are visible in twenty-four to forty-eight hours. The deep colonies are spherical, granular, and of a pale brownish colour, darker at the centre than at the periphery. The superficial colonies are at first round and almost transparent; rapidly spreading, so that they attain a diameter of 3 mm., their margins become irregular and surface ridged, or ringed, granular, opalescent in colour, and much thicker at the centre than at the periphery. On a gelatin streak a copious white, shining growth develops, the margins of which are irregular and crenated (fig. 49), and in old cultures the medium becomes milky. In a stab-culture a white growth develops along the line of inoculation with one or more gas bubbles, and the gelatin is not liquefied. On agar and blood serums a thick, moist, shining, greyish layer forms. There is abundant formation of gas in a stab-culture in sugar-agar, and in gelatin shake cultures (fig. 50). On acid potato it forms a straw-yellow or brownish-yellow growth, but if the potato be not fresh and acid in reaction the growth may be colourless. Milk is a good cultivating medium, and is curdled in twenty-four to seventy-two hours. This curdling appears to be due not to an enzyme,

but to the formation of acid, a considerable amount of which

is produced during its growth, chiefly lactic, which can be demonstrated by growing on litmus sugar-agar, or in litmus milk. The gas which is produced in culture media consists of carbon dioxide and hydrogen. In broth it produces a general turbidity without film formation, and the culture gives the indol reaction on the addition of a nitrite in twenty-four to forty-eight hours.

The thermal death-point of the organism, according to Weisser and Sternberg, is 60°C . with an exposure of ten



FIG. 50.—COLON BACILLUS. GELATIN SHAKE CULTURE
SHOWING GAS PRODUCTION.

minutes. The *bacillus coli communis* will grow freely in a slightly acid medium, and in media containing as much as 0.15 per cent. of carbolic acid. As mentioned before it is a more resistant organism than the *bacillus typhosus*.

Its pathogenic action is very varied. Introduced into the circulation in guinea-pigs it usually causes death in one to three days. Introduced into the peritoneal cavity in large amounts it produces death apparently from a toxæmia.

There are undoubtedly a large number of varieties of the

colon bacillus, and it is becoming customary to speak of the group of colon bacilli rather than to regard it as a single species. For example, Refik¹ describes five types or varieties of the normal colon bacillus isolated from water, characterised as follows :—

Type A.—Ferments lactose and glucose, coagulates milk, but does not give the indol reaction.

Type B.—Ferments lactose and glucose, does not coagulate milk, but gives the indol reaction.

Type C.—Ferments lactose and glucose, does not coagulate milk, and does not give the indol reaction.

Type D.—Does not ferment either lactose or glucose, coagulates milk, but does not give the indol reaction.

Type E.—Does not ferment either lactose or glucose, does not coagulate milk, and does not give the indol reaction.

All these varieties are motile, and have as common characters :—

1. The typical growth on potato.
2. A small number of flagella (not more than eight).
3. Develop more rapidly and more profusely on all the culture media than the *bacillus typhosus*.
4. Grow well in Fränkel's modification of Uschinsky's fluid (in which the *bacillus typhosus* does not grow at all).

It will be seen that these varieties approach nearer and nearer to the typhoid bacillus in their characters, and the question has been seriously discussed whether the colon bacillus may not under certain conditions become altered, and assume all the characters of the typhoid bacillus. Roux and Rodet came to the conclusion that this was the case, and that the colon bacillus, when grown in sewage, &c. might assume a pathogenic character, and give rise to a disease which is clinically indistinguishable from enteric fever. Klein in a long series of experiments, in which he grew the colon bacillus and the *bacillus typhosus* under similar con-

¹ *Ann. de l'Inst. Pasteur*, x. 1896, p. 242.

ditions side by side in various soils, and by passing through animals, was unable to effect any transformation of one form into the other; each organism retained unimpaired its differential characters.

It is most important that the colon bacillus and the *bacillus typhosus* should be distinguished from each other, and the differences between them are therefore given in the following table:—

—	Bacillus typhosus	Colon bacillus
Morphology.	Well-marked rod, five or six times as long as broad, with longer individuals and thread forms (involution forms)	Short rod, two or three times as long as broad; often so short as to be merely ovoid. Longer individuals occur and also involution forms, but not to such an extent as in typhoid
Flagella	Flagella long and wavy; 10-20	Flagella shorter than those of typhoid; 2-10
Motility	Actively motile	Feebly motile
Colonies, gelatin plates, 22° C.	Small, roundish, granular, brownish by transmitted light	Round or oval, brownish in colour. The surface colonies grow as a greyish expansion with furrowed surface and crinkled margins. Much larger than the typhoid colonies
Gelatin surface culture, 22° C.	Greyish-white growth, small in extent and almost confined to the needle track. Medium remains clear (fig. 46)	Greyish-white growth, spreading widely from needle track and nearly covering surface of medium, with crenated edges (fig. 49). Medium usually becomes cloudy near the growth
Gelatin 'shake' culture	No formation of gas	Many gas bubbles form (fig. 50)
25 per cent. gelatin kept at 37°C. for 48 hours (Klein)	The melted gelatin becomes uniformly turbid	The melted gelatin remains clear with a thick pellicle on the surface
Stab-culture in sugar-agar	No formation of gas	Many gas bubbles form
Acid potato.	Greyish, thin, invisible film	Straw-coloured, or yellowish-brown, thickish, slimy growth
Milk	No coagulation	Curdled in from 1 to 3 days
Broth	General turbidity, no film formation. No indol reaction	General turbidity, no film formation. Gives the indol reaction on the addition of a nitrite in from 24 to 48 hours

—	Bacillus typhosus	Colon bacillus
Broth containing 0·30 per cent. phenol	No growth . .	Grows well
Broth containing formalin (1 : 7000)	No growth . .	Grows well
Pfeiffer's reaction with typhoid serum	Positive . .	Negative
Bordet-Durham reaction with typhoid serum	Positive . .	Negative

These are, of course, the characters of the typical organisms, but considerable variation is met with in the case of the colon bacillus, not nearly so much in the *B. typhosus*.

The colon bacillus being such a variable organism, and capable of producing so many different pathological lesions, has undoubtedly been described under a variety of names and circumstances by different observers.

The following are some instances of this :

1. The *bacillus lactis aerogenes* found in the intestinal tract of infants by Escherich.
2. *Bacillus cavicida* of Brieger.
3. *Bacillus neapolitanus* described by Emmerich in cases of cholera.
4. Gas-forming bacilli described by Laser¹ and Gärtner.²
5. *Bacillus enteritidis* of Gärtner.³
6. Bacillus of the Middlesbrough pneumonia (Klein).
7. Aërobic bacillus of malignant œdema (Klein).
8. Bacilli found in cases of gastro-enteritis produced by meat-poisoning at Portsmouth (Klein).

All the above organisms closely resemble the colon bacillus, and may be merely varieties of it.

In man the colon bacillus is associated with a number of

¹ See *Centr. f. Bak.* xiii. 1893, p. 217. ² *Ibid.* xv. 1894, pp. 1 and 276.

³ See Klein, *Micro-organisms and Disease*, 1896, p. 224.

important pathological processes. It is usually the organism causing the peritonitis¹ which is due to infection from the intestine, as in hernia with obstruction or perforation, in ulceration of the bowel and enteritis, in cancerous growths, and trouble about the appendix and biliary canals and gall-bladder. The exudation in these cases is often characteristic; at first it is clear and greenish, it then becomes greenish-yellow, thin, semi-opaque and foul-smelling, and finally purulent. An important point is that the colon bacillus may pass through the intestinal wall when it has been damaged, as by strangulation, but not perforated. It is a pyogenic organism, and has been met with in ischio-rectal abscesses. It is also probably the cause in some instances of the pneumonia and pleurisy occurring after peritonitis, for it has been obtained from the lung and pleura in these conditions. Cystitis and infections after urinary operations are also commonly due to the colon bacillus, and Albarran and Mosny have prepared an antitoxin, by injecting animals with filtered cultures, which they believe will be useful not only as a curative measure, but also as a vaccine before operation to prevent infection.²

CLINICAL EXAMINATION.

1. The appearance and odour of the pus are often characteristic. Cover-glass specimens of the pus show small bacilli, which are decolourised by Gram's method.

2. Cultivations and plate cultures of agar and gelatin should be made, and the likely growths tested by subcultures on the various media.

3. Isolation by Parietti's method may also be attempted.

¹ 'Lettsomian Lectures on Peritonitis' (F. Treves), *Brit. Med. Journ.* 1894, i. p. 229 *et seq.* (Full Refs.)

² For literature on the Typhoid and Colon Bacilli, see *Science Progress*, ii. 1894, p. 27. *Bibliog.* (Buckmaster).

CHAPTER XI.

ASIATIC CHOLERA—SPIRILLUM OBERMEIERI—SPIRILLUM METSCHNIKOWI—SPIRILLUM OF FINKLER AND PRIOR—SPIRILLUM TYROGENUM—SPIRILLUM RUBRUM.

ASIATIC CHOLERA.

THE bacteriological study of Asiatic cholera may be said to date from the researches of Koch, who was sent by the German Government in 1884 to investigate the disease in Egypt and India. He described an organism met with in the intestine and in the dejecta which he believed to be the specific contagium, and called by him from its curved shape the 'Comma Bacillus.' This name is a misleading one, for the organism is not shaped like a printer's comma, but is a curved rod, and seems to belong to the group of spirilla; however, ever since it has been commonly known as Koch's comma bacillus.

SPIRILLUM CHOLERÆ ASIATICÆ.

Morphology.—Curved rods with rounded ends 1 to 2 μ in length, sometimes forming half a circle, sometimes united in pairs forming an S-shaped curve (fig. 51). It is more or less abundant in the intestine and in the alvine discharges, especially in the rice-like flakes, but is not found in the blood, organs, or tissues. In the rice-like flakes it is frequently so numerous that in a cover-glass specimen the 'commas' appear in masses crowded together and lying parallel to each other. It stains well with the ordinary

anilin dyes, especially with dilute carbol-fuchsin, but is decolourised by Gram's method.

Under cultivation in bouillon or in a hanging-drop specimen it is found that the organism develops into a longish spiral filament, and the commas are therefore regarded as resulting from the breaking up of a spirillum; but if the conditions of growth are very favourable multiplication may be so rapid that the curved rods or commas alone are produced, the organism dividing before it has time to grow into

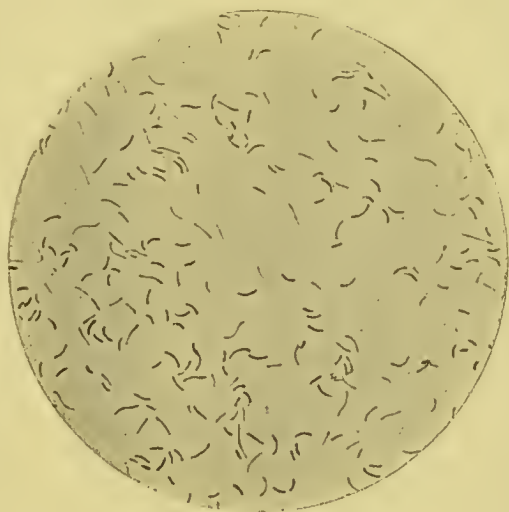


FIG. 51.—Koch's SPIRILLUM OF CHOLERA. $\times 1000$.

a spiral. It is actively motile, and possesses a single terminal flagellum at one end only, but there is some variation in this respect. Spores are not formed, though in old cultures Hueppe has described bodies which he believes to be arthrospores. In such cultures the bacilli lose their regular form, and swollen and distorted involution forms are seen. The Koch's spirillum is aerobic and facultative anaerobic, and grows well on the ordinary culture media from 20° to 37° C.

According to Frankland, although it grows readily in an

atmosphere of hydrogen, it does not develop in one of carbonic acid gas. In gelatin plates at 22° C. small cream-coloured colonies appear in about twenty-four hours, soon accompanied by liquefaction, so that in two or three days the plate becomes pitted. Microscopically, the young colonies are rounded with irregular margins, cream-coloured, and coarsely granular.

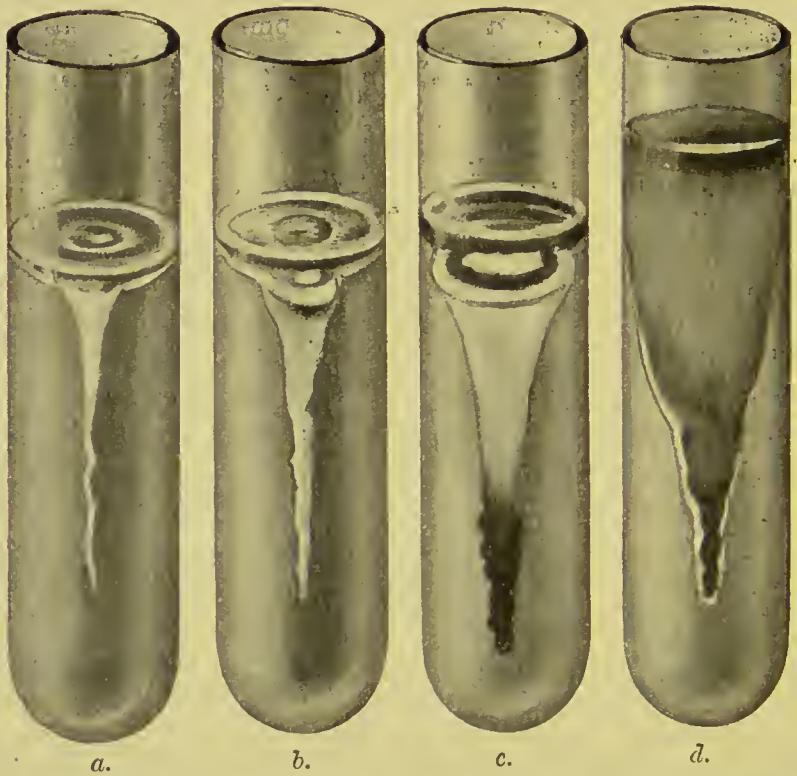


FIG. 52.—FOUR-DAY-OLD STAB-CULTURES IN GELATIN OF *a.* CHOLERA, *b.* SPIRILLUM METSCHNIKOWI, *c.* SPIRILLUM TYROGENUM, AND *d.* SPIRILLUM OF FINKLER AND PRIOR.

In stab-cultures development occurs all along the stab as a whitish, opaque, punctate growth, thicker above than below. Liquefaction commences about the second day and progresses slowly; in the early stage it is confined to the surface, and looks like a little bead or air bubble (*a*, fig. 52), but in a

fortnight or so the greater part of the gelatin may be liquefied. Liquefaction varies greatly both in rate and extent in different cultures and stocks; in some old laboratory cultures it may be almost or quite absent. On surface agar a thick, moist, shining, greyish growth quickly develops with more or less crenated margins. On blood serum much the same growth occurs with slow liquefaction. On potato at 37° C. a thin brownish layer is formed and broth becomes turbid, and a film forms on the surface. Peptone water, or Dunham's modification of it, is a good cultivating medium and becomes turbid, especially in the upper layers. In milk it multiplies rapidly with curdling; neutral litmus sugar-agar is reddened from the production of acid, but no gas is produced under cultivation. An important characteristic of the cholera spirillum is the rapid formation of indol in considerable quantity, and the reduction of nitrates to nitrites, especially in peptone water. This forms the basis of the important cholera-red reaction; two or three drops of pure sulphuric acid added to a peptone-water culture, eight to twelve hours old, give a pink colour, and the colour is intense when the culture is two to three days old. The reducing action of the cholera spirillum can also be shown by growing in litmus broth, which becomes decolourised (Cahen's test).

The cholera spirillum retains its vitality in cultures for months. It can multiply in water and on the surface of moist linen, but rapidly dies on complete desiccation. Its thermal death-point, according to Sternberg, is 52° C. with an exposure of four minutes; according to Kitasato, 55° C. in about ten minutes. It is readily destroyed by the ordinary antiseptics—hydrochloric acid 1 in 1300, carbolic acid 1 in 400, mercuric chloride 1 in 10,000, the time of exposure being two hours.

In some experiments by Dempster¹ it was found that the comma bacillus lived three to five days in dry soil, only one

¹ *Med. Chirurg. Trans.* lxxvii. 1894, p. 263.

day in an artificially dried soil, while it lived twenty-eight to sixty-eight days in moist soil. In peat, however, it was invariably dead within twenty-four hours. In sterilised salt solution (0·75 per cent.) the comma bacilli were alive on the 159th day, and in fresh urine (sterilised) they lived fourteen days at 37° C. and twenty-nine days at 22° C.

In sterilised distilled water the cholera spirillum usually rapidly dies, within twenty-four hours as a rule. The addition of sodium chloride greatly increases the length of time it may remain alive, a survival of five or six weeks having been recorded. In ordinary sterilised potable waters it may survive many months. In unsterilised potable waters its survival is greatly influenced by the presence of salts; in some cases it dies out rapidly; in others, especially in those containing a large proportion of salts, it may remain alive for a long time. In sterilised sewage the cholera spirillum multiplies and survives for months, in unsterilised sewage it probably rapidly succumbs.

Brieger in 1887 obtained cadaverin and putrescin and two other basic bodies from cholera cultures. Brieger and Fränkel isolated a tox-albumin, and Gamaleia a ferment-like body. Hueppe believes that the cholera poison is a tox-albumin found in the culture medium, but that immunising substances are derived from the bacterial cells.

Rontaler compared the chemical products of the ordinary and the Massowah cholera spirilla, and could find little difference.

Wesbrook¹ investigated the toxins produced by the Koch spirillum in different media. From alkali-albumin cultures he isolated deutero-albumose and traces of proto-albumose, from cultures in eggs a mixture of proteid bodies which it was impossible to separate, from asparaginate of sodium (Uschinsky's medium) a substance which gave a feeble xanthoproteic, but not the biuret reaction, and from the peritoneal exudation

¹ *Ann. de l'Inst. Pasteur; Journ. of Path. and Bact.* iv. 1896, p. 1.

of inoculated guinea-pigs a substance which, although it gave a slight xanthoproteic reaction, did not appear to be either deutero- or proto-albumose. This observer also found aërobic cultures of the cholera spirillum much more toxic than anaërobic ones.

Metschnikoff¹ and co-workers have demonstrated the existence of a soluble cholera poison in a very ingenious manner. Little sacs of collodion of 2 c.c. to 3 c.c. capacity were sterilised, filled with peptone solution, and inoculated with the cholera spirillum. The sac having been closed is introduced into the peritoneal cavity of a guinea-pig, which dies in three or four days.

By growing the cholera spirillum in a shallow layer with free access of oxygen in a peptone-gelatine-salt medium, Metschnikoff and co-workers have obtained a toxic fluid after three or four days' growth. During growth the fluid becomes concentrated to about one-eighth by evaporation. After filtration 0.25 c.c. kills a 300-gram guinea-pig in eighteen hours. Goats having been inoculated with increasing doses of this toxin, commencing with 10 c.c. and reaching 200 c.c., in six months, become immunised and yield an antitoxic serum, 1 c.c. of which will neutralise four times the lethal dose of toxin. Metschnikoff had previously found that young suckling rabbits suffer from an intestinal cholera when fed with cultures, so that they were able to test the effect of their cholera antitoxin in preventing intestinal cholera. Experiment showed that of the treated rabbits 51 per cent. survived, of the untreated only 19 per cent. These experiments are of interest from the point of view of the antitoxic treatment of the disease in man.

The blood serum of an animal immunised by injections of the cholera spirillum gives a typical 'Widal' reaction with recent cultures of the cholera spirillum—that is, it stops motility and causes aggregation of the spirilla. Achard and

¹ *Ann. de l'Inst. Pasteur*, x. 1896, p. 257.

Bensaude¹ state that the reaction can be obtained with blood serum of cholera patients, sometimes as early as the first day of the disease.

The relation of the cholera spirillum to the disease has been a very vexed question in the past, but the outcome of the voluminous researches which have since been made is to confirm Koch's work.

None of the lower animals suffer from or contract a disease in any way comparable to Asiatic cholera, so that the test of animal experiments cannot be applied. By first neutralising the acidity of the gastric juice by an injection of sodium carbonate solution into the stomach, then diminishing peristalsis by an injection of tincture of opium into the peritoneal cavity, and finally injecting a broth culture of the cholera spirillum into the stomach, Koch succeeded in inducing in guinea-pigs a condition somewhat similar to cholera in man—namely, indisposition with falling temperature, weakness of the extremities, and death in forty-eight hours. Post-mortem, the small intestine was congested and filled with a watery fluid containing large numbers of the cholera spirillum. Injected into the peritoneal cavity of mice and rabbits, it usually produces death. There are also one or two cases on record where individuals have apparently suffered from a genuine choleraic attack, with abundance of spirilla in the dejecta, as a result of the accidental ingestion of cholera cultures.

That the cholera spirillum is associated with the disease seems to be beyond any doubt, and so constant is its presence in true cholera that all investigators, even those who were once most opposed to Koch's views, rely on its detection for the bacteriological diagnosis. The matter, however, has recently become somewhat complicated owing to the detection in various natural waters of pathogenic spirilla which, although not identical with the cholera spirillum of Koch, resemble it so closely that it is difficult to classify them as anything but

¹ *La Presse Médicale*, 1896, September 26.

varieties of the cholera spirillum. In certain epidemics in India variations have also been noted in the cholera spirilla which have been isolated. Sanarelli¹ isolated from the Seine and Marne thirty-two spirilla, of which four were almost indistinguishable from cholera, except that they were only slightly pathogenic, but by passage through a series of animals their pathogenic power was much enhanced. Sanarelli believed that these were the descendants of true cholera spirilla, which had gained access to the rivers during some previous epidemic of cholera. Dunbar similarly, from the Elbe and Rhine, &c. isolated a number of spirilla which could not be distinguished from the cholera spirillum (*Spirillum* Elwers). It was afterwards noticed that some of these under certain conditions of oxidation and temperature became phosphorescent,² but Rumpel³ has also found that cultures of the genuine cholera spirillum may exhibit phosphorescence, so this cannot be used as a differential character for the separation of non-choleraic forms. Neisser isolated a spirillum, which he termed the *vibrio Berolinensis*, which agreed with the cholera spirillum in every particular except that the colonies in a gelatin plate were invisible to the naked eye in forty-eight hours. Heider found in the Danube a spirillum, named by him the *vibrio Danubicus*, which resembled the cholera spirillum closely, but its colonies were somewhat different, and it was more actively pathogenic to mice. Ivanoff similarly obtained a spirillum which could only be distinguished from cholera by the finer granulation of its colonies and more distinct spiral form. Lastly, there is the *spirillum Massowah*, isolated from an epidemic of cholera at Massowah, in India, which differs from the Koch spirillum in having two terminal flagella at each end. Cunningham has also described several spirilla differing slightly from the cholera spirillum.

¹ *Ann. de l'Inst. Pasteur.*

² *Centr. f. Bak. 1te Abth.* xviii. 1895, p. 424 (Kutscher).

³ *Munch. Med. Wochenschr.* 1895, No. 3.

The isolation of all these spirilla, differing slightly from the Koch spirillum yet resembling it in many points, raises the important question whether they are merely varieties of the Koch spirillum, or distinct species; in the latter case it is evident that a bacteriological examination would be of little value in the diagnosis of cholera unless very prolonged and thorough, conditions which would necessarily defeat the whole aim of this method. Fortunately, however, by the use of two tests a good deal of light has been thrown on this question. The first of these is known as 'Pfeiffer's specific reaction.' This reaction depends on the specific action of the serum of an immunised animal. A mixture of an emulsion of the organism to be tested, with a small quantity of serum from a highly immunised animal, is injected into the peritoneal cavity of a normal guinea-pig. The fluid in the peritoneal cavity is then examined microscopically half to one hour after the injection, and if the reaction be positive the organisms will be found in all stages of degeneration, being mostly converted into spherules. In this case, according to Pfeiffer, the organism is to be regarded as belonging to the same species as that by means of which the immunisation of the animal, from which the blood serum was obtained, was carried out. If, on the other hand, the reaction be negative, the organisms are unaffected after being in the peritoneal cavity for an hour or so, and the organism is then considered to be different in species from that used for the immunisation. Pfeiffer's reaction has been chiefly made use of to differentiate the various cholera-like spirilla and the typhoid and colon bacilli from each other. The other test may be termed the 'Bordet¹-Durham'² reaction (Klein), though Gruber's name ought also to be associated with it.

In this test an emulsion of the organism is mixed with the diluted serum of an immunised animal. If the reaction

¹ *Ann. de l'Inst. Pasteur*, 1895, p. 462.

² *Journ. Path. and Bact.* iv. 1896, p. 13.

be positive a hanging-drop specimen of this mixture will show the organisms deprived of their motility, and for the most part aggregated into clumps. If the mixture be placed in a narrow test-tube, in an hour or so the fluid will be found to have become clear, the organisms forming a flocculent precipitate at the bottom. Now to apply these two tests to the spirilla in question. In the first place, each of the organisms gives a complete positive reaction to both tests with its own serum; this, of course, is only to be expected. Pfeiffer found that, using his reaction, the variety *Ivanoff* gave a positive reaction with cholera serum, and Durham found that *Ivanoff* and *Berolinensis* reacted completely with cholera serum. Conversely, positive reactions with cholera spirilla were obtained with *Massowah*, *Danubicus*, and *Elwers*' sera, while *Massowah*'s and *Elwer*'s react completely to each other. From these considerations it would therefore seem probable that the spirilla under discussion, viz. *Sanarelli*, *Elwers*, *Berolinensis*, *Danubicus*, *Ivanoff*, and *Massowah*, are varieties of the Koch spirillum.

The variability of the Koch spirillum is further shown by some experiments of Klein.¹ While investigating the relation of oysters to enteric fever this observer made a number of experiments with the cholera vibrio. He ascertained that the cholera vibrio could retain its vitality for at least fourteen days in unsterilised sea-water, while from the interior of oysters, kept in water infected with the vibrios, it was obtained up to nine days after infection. Some of the vibrios obtained from the water and from the oysters showed, however, marked variation from the parent stock—variations in the rate of liquefaction, in the curdling of milk, in the indol reaction, &c.; and five more or less definite varieties were obtained, two of which did not respond to Pfeiffer's test, although originally derived from a

¹ Local Government Board Report, 'On Oyster Culture in relation to Disease,' 1896, p. 135.

genuine cholera vibrio responding to all the tests, including Pfeiffer's. Klein, therefore, says: 'It seems to me that in the case of Pfeiffer's test, as in many other instances, though a positive result may help to establish and to prove the proposition, a negative result has much less value as a basis of inference!'

Haffkine has prepared a vaccine against cholera from cultures of the Koch spirillum (see Appendix) which seems to be efficacious in preventing the disease. For example, a number of labourers were inoculated during an epidemic, and among the inoculated the mortality was only 2·25, whereas among the uninoculated it was nearly 19 per cent.

CLINICAL DIAGNOSIS.

This is based on a microscopical examination and culture methods.

1. From one of the whitish, slimy, rice-like flakes in the evacuations or the intestine cover-glass specimens are prepared, stained in Löffler's blue, washed, dried and mounted. If upon a microscopical examination large numbers of curved rods lying in groups parallel to each other are observed, the diagnosis of Asiatic cholera may be made with some degree of certainty. Koch states that this is so in quite half the cases, especially the acute ones.

2. A set of gelatin plates and a set of agar plates should be prepared from other rice-like flakes. In the case of the agar plates they should be prepared by pouring the melted agar into the Petri dishes, allowing it to set, and then smearing the flake over the surface. The plates are incubated at 22° C. and 37° C. respectively. In the gelatin plates the characteristic colonies of the cholera spirillum should be recognisable in about twenty-four hours, in the agar plates in from twelve to sixteen hours. The likely colonies should be examined microscopically and peptone-water cultures prepared from them.

3. With other rice-like flakes several peptone-water cultures should be prepared and incubated at 37° C. In eight to ten hours the upper layers of the fluid should be examined microscopically for the presence of commas, and gelatin plates and

subcultures in peptone water also made by inoculating from the surface layer of fluid. The peptone-water culture may then be tested for the presence of indol by carefully adding a few drops of pure concentrated sulphuric acid. In cases of Asiatic cholera the indol reaction can be obtained as early as eight hours after inoculation.

4. To any doubtful commas Pfeiffer's test may be applied, inoculating the commas together with some cholera serum intraperitoneally into a guinea-pig, and examining the peritoneal fluid microscopically half an hour afterwards.

5. The Bordet-Durham test may likewise be applied to the commas, observing the precipitating and agglutinating action of an experimental cholera serum *in vitro* and microscopically in hanging-drop specimens. This can be done not only with cultures from, but also directly with, the suspected rice-like flakes, provided they contain numerous commas, an emulsion in sterile water being made.

6. If the case has lasted any time the Widal method of diagnosis may be applied—viz. testing the action of the blood or serum of the patient on a known stock of Koch's spirillum. As in typhoid fever, the serum causes cessation of motility and agglutination or clumping.

7. Cover-glass specimens of cultures are best stained with carbol-fuchsin, diluted with water (1 : 4).

SPIRILLUM OBERMEIERI.

This, the spirillum of relapsing fever, discovered by Obermeier in 1873, is constantly found in the blood during the febrile paroxysms. It occurs as a long, slender, spiral filament, not unlike the *spirillum rubrum*, 20 to 30 μ in length, and actively motile. It has not been cultivated up to the present time. Healthy persons inoculated with blood containing the organisms suffer from the disease, and the spirilla are met with more or less numerous in their blood during the febrile paroxysms, but disappear during the intermission. Vandyke Carter, Koch, and Soudakowitch have successfully

inoculated monkeys, which seem to be the only susceptible animals. They suffer from a febrile condition, during which numbers of the spirilla can be seen in the blood, but relapses do not occur.

CLINICAL EXAMINATION.

In a case of suspected relapsing fever a drop or two of blood should be obtained from a prick in the finger, and several preparations examined microscopically in the fresh state. The spirilla will be readily recognised if present, but several fields should be examined, as they are sometimes scanty.

SPIRILLUM METSCHNIKOWI.

Isolated by Gameleia from the intestinal contents of chicken dying from an infectious gastro-enteritis which occurs in certain parts of Russia. The disease, although resembling chicken cholera in some respects, is quite distinct from the latter. This spirillum forms curved rods and spiral filaments, generally slightly shorter, thicker, and more curved than the Koch spirillum. It is decolourised by Gram's method, and is best stained with weak carbol-fuchsin. It is readily cultivated and is aërobic and facultative anaërobic. In gelatin plates it forms small whitish colonies, visible within twenty hours, which increase in size, and in two or three days produce marked areas of liquefaction. In a stab-culture in gelatin a whitish, granular growth occurs along the line of puncture with liquefaction, much like that of the Koch spirillum, but the rate of growth and the liquefaction are more rapid (*b*, fig. 52). On surface agar a thick cream-coloured layer develops. On potato the growth is brownish, while milk is coagulated. It grows freely in broth and peptone water, the fluid becoming uniformly turbid, and a slight film forming on the surface, and these cultures give a marked indol reaction on the addition of sulphuric acid alone, in this respect

resembling the Koch spirillum. It is pathogenic to chicken, but not to rabbits or mice except in large doses. Abbott¹ has isolated a pathogenic spirillum from the Schuylkill River, Philadelphia, which resembles the *vibrio Metschnikovi* closely, and is probably identical with it.

SPIRILLUM OF FINKLER AND PRIOR.

Isolated from the stools in certain cases of cholera nostras, but its aetiological significance is doubtful. It occurs as short, thickish, curved or straight rods, and sometimes as spiral filaments. It is aërobic and facultative anaërobic. It does not form spores, and does not stain by Gram's method. In a gelatin stab-culture a yellowish growth forms with rapid liquefaction (*d*, fig. 52). On agar a thick, slightly brownish, moist layer develops. On potato a slimy brownish growth occurs even at room temperature. It grows in broth and peptone water, producing a general turbidity. It does not as a rule give the indol reaction with sulphuric acid alone, but the ordinary laboratory cultures after three to four days' growth occasionally give a slight reaction. It is stated to be pathogenic to guinea-pigs.

SPIRILLUM TYROGENUM.

Obtained by Deneke from old cheese, and frequently spoken of as Deneke's spirillum. It forms curved rods and spiral filaments somewhat closely resembling the Koch spirillum. It grows well on the ordinary culture media at room temperature, but development is usually slight or absent at 37° C. In a gelatin stab-culture a yellowish growth occurs with liquefaction, which is much more rapid than that of the Koch spirillum, but less so than that of the Finkler-Prior spirillum (*c*, fig. 52). On agar a thinnish,

¹ *Journ. of Exper. Med.* i. 1896, p. 419.

brownish, somewhat membranous and coherent layer slowly develops at room temperature. On potato a yellowish growth occurs. It is stated to be slightly pathogenic to guinea-pigs.

SPIRILLUM RUBRUM.

A chromogenic spirillum obtained by Koch from the putrefying tissues of a mouse. In a gelatin stab-culture a dark red growth slowly develops along the line of puncture; at the surface, however, the growth is colourless. In broth at 37° C. it grows freely, producing a general turbidity with a red deposit at the bottom of the tube; there is no film formation. In such a broth culture large numbers of typical spirillar filaments can be seen, which are thin and delicate, of varying length, and actively motile. It is non-pathogenic.

CHAPTER XII.

BUBONIC PLAGUE—CHICKEN CHOLERA—SWINE FEVER—MOUSE
SEPTICÆMIA—MOUSE PLAGUE.

BUBONIC PLAGUE.

THE bubonic plague was epidemic throughout Europe during the Middle Ages; in England in the fourteenth century it appeared as the Black Death, and in the seventeenth century as the Great Plague of London; numerous other lesser visitations have also been recorded. It seems to have been almost always endemic in Persia and Syria. One peculiarity about this disease is the manner in which it completely disappears from a district which it has decimated, to reappear again after considerable lapses of time; this has happened not only in Europe, but also in Persia, Syria, India, and China.

It is a severe febrile disease accompanied by delirium and great depression, which rapidly passes on to the typhoid state. If the patient lives, hæmorrhages may occur under the skin and from the stomach, bowel, and kidneys. On the second or third day the glands in the axilla, inguinal, and other regions become enlarged, whence the disease derives its name.

¹The bacillus of bubonic plague was first described by Kitasato early in 1894. He observed it in the blood of living patients, and in the blood, buboes, and organs of those who had died of the disease. In his preliminary communica-

¹ The following account of the bacillus is largely taken from a paper by the writer in the *Transactions of the British Institute of Preventive Medicine*, vol. i. 1897.

tion¹ (which has not, so far as I know, been followed by a subsequent one) he describes the plague bacillus as a rod with rounded ends, staining readily and showing marked polar staining, but slightly motile, and sometimes apparently capsulated. It grew freely on blood serum and agar at 37° C. as a greyish moist film, and without liquefying the former. In beef broth its growth made the medium somewhat cloudy. In cultivations long chains of bacilli occurred, the individual bacilli of which were so short as to be almost coccoid. Owing to the high temperature prevailing at Hong Kong, the characters of the growth on gelatin could not be ascertained. On potato there was a slight growth as a greyish film at 37° C.; spores were not observed. It was pathogenic for mice, rats, guinea-pigs and rabbits. Later in the same year (1894) Yersin² was commissioned by the French Government to investigate the outbreak of bubonic plague at Hong Kong. He described the bacillus met with in the buboes as short and thick with rounded ends, staining readily, but not by Gram's method, showing polar staining, and sometimes apparently encapsuled. On gelatin it formed whitish, transparent colonies, some of the colonies developing much more rapidly than others, and containing bacilli which are less virulent than in the slower-forming colonies. In broth the growth was very characteristic: the fluid remained clear, while a grumous deposit formed on the sides and bottom of the tube consisting of chains of very short bacilli, among which swollen involution forms occurred. It killed mice in one to three days, rats and guinea-pigs in two to five days, rats also dying when fed with plague material, and was pathogenic for rabbits, and in large doses for pigeons. Under cultivation the bacillus readily loses its virulence. Aoyama³ also gives an account of the bacillus of bubonic plague based on the

¹ *Lancet*, 1894, ii. p. 428.

² *Ann. de l'Inst. Pasteur*, viii. 1894, p. 662.

³ *Mittheil. aus der Med. Facult. der kaiserl. Japan. Universität*, Bd. iii. No. ii. p. 115.

descriptions of Kitasato and Yersin. He considers that the bacilli met with in the blood by Kitasato, and stated by that observer to stain by Gram's method, are not identical with those present in the glands. The bacilli did not liquefy blood serum. Yersin, Calmette, and Borrel,¹ in some work on immunisation, found that by passage through a series of animals they could obtain a 'fixed virus' which killed mice in two days, young guinea-pigs in two to three days, and rabbits in three days. They were unable to obtain toxic substances from the cultivations, and for preparing an anti-toxin were obliged to make use of attenuated cultures.

Zettnow² states that the bacillus of plague is non-motile, and although a thick capsule is apparently present, he was unable to demonstrate one. After twenty-one days in a gelatin stab-culture a snow-white growth is present.

The bacillus of bubonic plague on which the following description is based was isolated from a case which was under Dr. Manson's care at the Seaman's Hospital, Albert Dock. The material was received in two sterilised test-tubes, one containing a piece of the enlarged femoral gland, the other a small piece of the neighbouring femoral vein. An emulsion was made in sterile water with the piece of gland, and from it plate cultivations and cultivations on various media were prepared. A guinea-pig was inoculated with the remainder of this emulsion, and the piece of vein was inserted under the skin of another guinea-pig. The cultivations proved to be much contaminated, partly perhaps because, as pointed out by Aoyama, other organisms occur in the buboes, and partly because the material was not manipulated until a lapse of three or four days after death. For the latter reason no direct microscopical examination of the tissues was made. Before any likely organism had been isolated from the cultures, the guinea-pig inoculated with the

¹ *Ann. de l'Inst. Pasteur*, ix. 1895, p. 589.

² *Zeitschr. f. Hygiene*, xxi. 1896, p. 165.

gland died, so their further examination was abandoned. The animal did not show any symptoms for two or three days; it then began to be sluggish, and to have a slight dis-

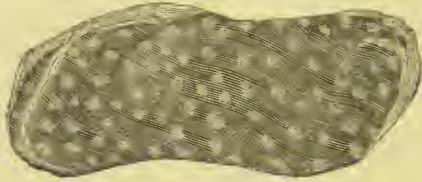


FIG. 53.—SPLEEN OF GUINEA-PIG INOCULATED WITH PLAGUE. (Nat. size.)

charge from the eyes, and on one occasion, on the fifth day, suffered from a curious attack—fell on its side, the heart's action became feeble, the respiration infrequent, shallow, and gasping, and there was every appearance of a speedy ending.

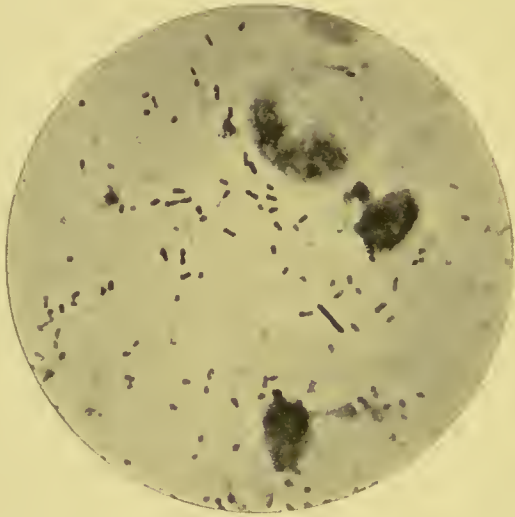


FIG. 54.—PLAGUE. SMEAR PREPARATION FROM SPLEEN OF INOCULATED GUINEA-PIG. $\times 1000$.

Recovery followed, however, and death did not take place until the seventh day, two days later.

The post-mortem appearances were: Hæmorrhages and

great œdema at the seat of inoculation, and great enlargement of the lymphatic glands in the groin. The most striking feature was the appearance of the spleen; it was much enlarged, four to six times larger than normal, deeply congested, and studded all over with yellowish points like miliary tubercles (fig. 53). Microscopical examination of the fluid from the seat of inoculation, of the organs and blood, showed the presence in enormous numbers of a short, thick, ovoid bacillus with rounded ends, generally in pairs, so short as to appear at first sight to be a large diplococcus. Cultivations were readily obtained on various media, and the following are the characters of the organism:—

Morphology.—A markedly pleomorphic organism. *In the animal body* it occurs for the most part as a short, almost ovoid rod, generally linked in pairs, measuring $2.3\ \mu$ by $1.7\ \mu$, but longer forms are to be seen here and there measuring as much as $5\ \mu$ (fig. 54).

In Cultivation.—The bacilli in young cultures (twenty-four to forty-eight hours) are so short as to be almost coccoid or slightly ovoid, on agar of about the same size as in the animal body, on gelatin, however, slightly smaller, but a few well-marked rods and even threads are always to be seen sparsely distributed in the preparations. In older cultures rod, thread, and involution forms occur more numerously.

In broth the organism forms chains of slightly ovoid bacilli, appearing at first sight like a streptococcus (fig. 55).

Motility.—The organism is non-motile.

Capsulation.—Sometimes in drop cultivations there is apparently a capsule, but I have failed to verify this by staining methods.

Blood-serum Cultures.—On blood serum it forms moist, smooth, shining, cream-coloured colonies or growths, slightly raised above the surrounding medium. The blood serum is not liquefied.

Agar Cultures.—Colonies are raised, round and cream-

coloured, finely granular, denser at the centre than at the margins. Margins regular. Size, 0·25 to 0·5 mm. in two days at 37° C.

Agar Cultures (Surface).—Forms a thick, opaque, moist, smooth, cream-coloured growth, the margins of which are usually markedly crenated. The growth is very sticky and adherent (fig. 56).

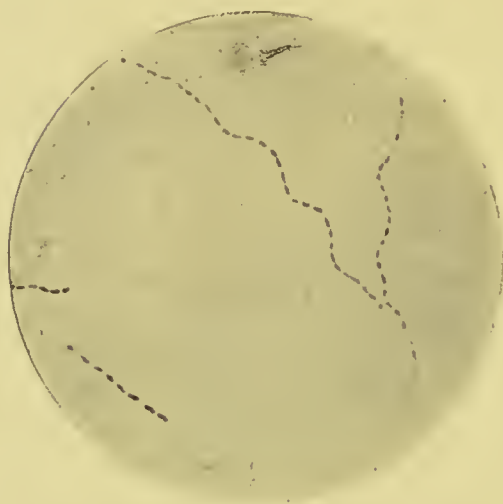


FIG. 55.—PLAGUE; 48 HOURS' BROTH CULTURE.
× 1000.



FIG. 56.—PLAGUE.
SURFACE CULTURE
ON GLYCERIN AGAR,
48 HOURS OLD.

Sugar-agar Cultures (Stab).—Grows to the bottom of the stab as a cream-coloured streak which does not diffuse into the surrounding medium. There is no development of gas.

Gelatin Cultures.—Colonies are whitish, filmy, finely granular with regular margins. Size, 0·1 to 0·25 mm. in five days at 22° C.

Gelatin Cultures (Surface).—Forms a thick, white, shining growth, with slightly irregular surface and margins, nearly confined to the inoculation track. The growth does not penetrate into the medium, nor does it render it cloudy. The growth is very adherent. No liquefaction.

Gelatin Cultures (Stab).—Delicate whitish, finely granular growth, developing to the end of the stab, and with little tendency to spread from the needle-track. The gelatin is not liquefied. Both in agar and gelatin cultures, fresh punctate growths sometimes develop in the original growth, simulating a contamination.

Broth Cultures.—Growth somewhat characteristic. For two or three days the broth remains perfectly clear, but a flocculent growth forms and gradually increases in amount on the bottom and sometimes on the sides of the tube. After some days the broth may become a little cloudy. Film formation very slight, or absent.

Potato.—No growth occurs on ordinary potato.

Milk.—Milk is not coagulated.

Indol Production.—With sulphuric acid alone a feeble indol reaction can be obtained with week-old broth cultures. With sulphuric acid and a nitrite a well-marked indol reaction can be obtained under the same conditions.

Acid Production.—With *neutral litmus agar* no acid or alkaline reaction. With *neutral litmus sugar-agar* a well-marked acid reaction after two days' growth.

Reducing Action on Methylene Blue.—Broth cultures reduce a weak solution of methylene blue.

Vitality of Cultures.—Cultures retain their vitality for at least a month, but the limit has not been determined.

Pathogenesis.—Guinea-pigs die in three to seven days; if in three days, the enlargement and nodulation of the spleen may not be marked. Mice die in two to three days, the spleen being much enlarged. Rats die in about a week after being fed with the organs of a guinea-pig which has died of

the disease. Occasionally a very small dose may fail to kill a guinea-pig.

Distribution in the Tissues.—The organism is met with in all parts of the body. At the seat of inoculation, in the glands and spleen it is extremely abundant, less so in the liver, lung, and kidney, not nearly so numerous in the blood. The chief changes observed in the tissues are cloudy swelling and hyperæmia, and numbers of minute hæmorrhages. The opportunity afforded for studying the bacteriology of bubonic plague in this country has fortunately remained unique.

Haffkine¹ has drawn attention to a stalactite sort of growth which occurs in undisturbed broth cultures (containing fat) of the plague bacillus. Within the first twenty-four hours little islands of growth appear near the surface, and from these flakes processes grow down, the liquid remaining clear. On somewhat desiccated agar large swollen involution forms occur resembling yeast cells, and the same may be met with in the tissues.

Kolle² states that large numbers of bacilli with rounded ends are found in the buboes, and also in the blood and spleen. The bacillus shows marked polar staining, but does not stain by Gram's method. It forms greyish growths on agar and gelatin, the gelatin not being liquefied. Spore formations did not occur, and cultures were killed by exposure to a temperature of 58° C. for several hours. Rats, mice, and guinea-pigs are susceptible, and rats and mice contract the disease by feeding on cultures of the bacillus. Rats and guinea-pigs could be immunised against the bacillus by inoculation with a culture which had been killed by heating to 65° C. for several hours.

Abel³ found that the plague bacillus is rapidly killed by desiccation at 30° C. over sulphuric acid. According to him the best disinfectants are corrosive sublimate 1-1000, and

¹ *Brit. Med. Journ.* 1897, i. p. 1461.

² *Deut. Med. Woch.* 1897, March 4. ³ *Centr. f. Bakt.* 1897, April 24.

chloride of lime 1-100 solutions. Solutions of carbolic acid of less than 5 per cent. were useless.

Gordon¹ states that the *bacillus pestis* possesses one or two spiral terminal flagella.

Haffkine has prepared an anti-plague vaccine. The plague bacillus is grown in a fluid medium, rich in fat, and with free aëration. When there is a copious stalactite growth, the broth is shaken to break it up, another is allowed to form and the process repeated, and in the course of a month half a dozen successive crops are obtained. The organisms are then killed by heating to 70° C. for an hour. This forms the vaccine fluid; for use it is shaken and 3 c.c. injected.

Lustig and Galeotti² have also devised a vaccine against the plague bacillus; it is prepared by growing the bacillus on the surface of agar in dishes for three days, scraping off the growth, and treating with 1 per cent. caustic soda solution. The fluid is then filtered through paper and precipitated with acetic or hydrochloric acid, or by saturation with ammonium sulphate. The precipitate is dissolved in a weak solution of sodium carbonate, and filtered through a Chamberland filter. This forms the vaccine fluid, which has the chemical characters of a solution of nucleo-proteids; it protects animals, and from the vaccinated animals an anti-toxic serum can be obtained. Yersin³ has immunised horses by injecting cultures of the plague intravenously, the injections being repeated with increasing doses. With the serum of such immunised horses he has treated twenty-six patients, with two deaths, a mortality of 7.6 per cent. The dose employed was 10 c.c., and a total of 30 c.c. usually sufficed for a case.

The spread of the disease is largely due to personal contact, and filth and insanitary conditions generally are powerful factors in favouring its development. Domestic animals are

¹ *Centr. f. Bakt.* 1te Abt. xxii. 1897, p. 170.

² *Brit. Med. Journ.* 1897, i. p. 1057.

³ *Ann. de l'Inst. Pasteur*, xi. 1897, p. 81.

also probably responsible in some cases in spreading infection, especially rats. Yersin, in a later paper, states that just previous to an epidemic of plague the rats die in large numbers, and then human beings are attacked. In the rats the specific organism is found, and flies may also convey the infection.

CLINICAL EXAMINATION.

1. Make cover-glass preparations from the blood and buboes, and stain with anilin gentian violet, and decolourise slightly in weak spirit. Mount and examine. Search for short rods in pairs, almost resembling diplococci, with a tendency to polar staining.

N.B.—There is very likely to be a mixture of organisms in the buboes.

2. Make cultures and plate cultivations from the buboes.

3. Inoculate a guinea-pig subcutaneously from a bubo. If it dies, investigate for the *bacillus pestis*.

4. Hankin¹ has recently described remarkable involution forms—spheres, and pear-shaped bodies—when the plague bacillus is grown on agar containing 2·5 to 3·5 per cent. of salt at 37° C. from twenty-four to forty-eight hours. So characteristic are these that he considers they form a means of diagnosis.

CHICKEN CHOLERA.

Chicken cholera is a disease of poultry characterised by profuse diarrhoea; its course may be very rapid and the bird found dead without having shown signs of illness. The organism is a very short rod, so short that it is almost ovoid, 0·6 to 0·8 μ in length, and 0·3 to 0·4 μ in diameter. It stains by the ordinary anilin dyes, but not by Gram's method, and the staining tends to be polar, so that Pasteur, who first investigated the disease, described it as a diplococcus. It grows freely on the various culture media from 20° to 38° C.; on agar

¹ *Centr. f. Bakt.* xxii. 1897, p. 438.

it forms a thick, moist, cream-coloured layer, on gelatin a shining, white, expansive growth without liquefaction. In broth it forms a general turbidity; on potato it does not grow very well. It is *aërobic* and *facultative anaërobic*, does not form spores, and is killed by a temperature of 50° C. in fifteen minutes. If dried it dies in a few days, but retains its vitality for a considerable time in damp earth or in water, and so infection is readily conveyed. Fowls die after subcutaneous, intramuscular, or venous inoculation and by feeding, the organisms being found abundantly in the blood. Post-mortem, the serous membranes may be inflamed and hæmorrhagic, the liver large and soft, and the intestine shows hæmorrhagic spots, and is sometimes ulcerated and contains a mucous fluid stained with blood. Other birds, pigeons, pheasants, sparrows, wild and domestic ducks, are also susceptible to the disease, and rabbits and guinea-pigs can be successfully inoculated; in the latter animal a local abscess sometimes forms instead of a general infection. By continuous cultivation with free access of oxygen the virus becomes attenuated, and Pasteur was able thus to prepare a vaccine which protected fowls.

The bacillus of chicken cholera seems to be identical with the bacillus of rabbit septicaemia of Koch, and with the bacillus of swine plague of Löffler and Schutz, and of Salmon and Smith.

Organisms have been described by Klein in fowl enteritis, grouse disease, &c. differing somewhat from the bacillus of chicken cholera.

SWINE FEVER.

Swine fever, or hog-cholera, is an infective disease of pigs, highly contagious, and causing considerable mortality. The duration of the affection is usually three to four weeks; the animals lie about, their temperature is raised, and they may suffer from cough and frequent respiration, and some lameness in the hind legs. Towards the end mucous diarrhœa is a prominent

symptom. Post-mortem, the large intestine is found to be ulcerated, the ulcers much resembling the typhoid ulcers of man, and according to Klein, pneumonia is commonly present, whence he termed the disease 'pneumo-enteritis.' MacFadyean, however, from his own experience and that of the Board of Agriculture, considers pneumonia very infrequent. There seem to be two or three varieties, if not species, of bacilli described by different observers in this disease. Klein describes the bacillus as a short rod with rounded ends, actively motile, readily stained with the ordinary anilin dyes, but not by Gram's method. It is aerobic, and does not liquefy gelatin. Cultures are much like those of chicken cholera.

Professor J. MacFadyean¹ has demonstrated the lesions occurring in swine fever and the micro-organism to which it is due. He describes the latter as a small bacillus, closely resembling in size and form the glanders bacillus. In ordinary cases of the disease it is frequently present in the large intestine and in the mesenteric glands; in very acute cases, though seldom or never demonstrated by microscopic examination, it is also present in small numbers in the blood. It is motile and aerobic, and can be cultivated in most of the ordinary artificial media, though its growth on gelatin is very meagre, and on potato it does not form a visible growth. In gelatin agar its growth is most characteristic, the colonies having a very distinctive cloudy aspect. At ordinary room temperature its growth is very slow, and it thrives best at blood-heat. Artificial cultures are easily destroyed by desiccation; it is also destroyed by exposure to a temperature of 58° C. for ten minutes. It is an obligatory and not a facultative parasite. The infection in natural cases is commonly caused by the ingestion of the bacilli voided with the faeces of animals previously attacked. In very acute cases, dying within two or three days after infection, there is nothing at the post-mortem examination to distinguish the lesions caused by this disease from those of acute gastritis and enteritis caused by an irritant chemical poison. In artificial cultures the organism retains its virulence through many successive generations. In the great majority of cases the

¹ 'Proceedings of the Path. Soc.' in *Brit. Med. Journ* 1896, ii. December.

lesions are very distinctive, and their most constant seat is the cæcum and colon. The commonest form of the intestinal lesion is a well-defined circular necrosis involving the whole thickness of the mucous membrane and occasionally extending to the wall of the bowel. A diffuse diphtheritic lesion comes next in frequency, being in reality a superficial necrosis with deposition of a thin layer of fibrinous exudate on the surface. All gradations are found between the well-defined circular necrosis and the diffuse diphtheritic lesion.

Although the lesions are very similar, swine fever has nothing to do with enteric fever of man, nor has any relation been established between it and ulcerative colitis, as has been suggested.

MOUSE SEPTICÆMIA.

This disease may be conveniently described here. Koch first obtained a minute bacillus by injecting putrefying material subcutaneously into mice. It seems to be identical with a bacillus found in swine erysipelas. The organisms are met with in large numbers in the blood and tissues of mice. They measure only 1μ in length, and occur in considerable numbers in the leucocytes. The bacillus stains well by Gram's method, and is stated by some writers to be motile. It grows readily, forming on agar extremely delicate, almost invisible colonies; in stab gelatin cultures after some time a delicate cloudiness radiates from the central puncture. From an agar culture the bacilli are somewhat larger than those found in the animal body, and form filaments. It is pathogenic for swine, rabbits, and mice.

MOUSE PLAGUE.

An organism producing an epidemic disease among mice, and isolated by Löffler. It forms small rods and filaments and is motile. On agar and potato it produces thick, moist, greyish-white growths; on gelatin, whitish growth without liquefaction. Cultures have been used for the extermination of field-mice, which in some districts are a great pest.

CHAPTER XIII.

PNEUMONIA AND INFLUENZA.

PNEUMONIA.

SEVERAL types of pneumonia must be recognised clinically, and are associated with different organisms. The pneumonia accompanying or complicating many of the specific fevers is usually of the catarrhal or lobular type, and may be set up by their respective organisms, as in diphtheria, enteric fever, influenza, &c. There is also the so-called 'septic pneumonia,' unfortunately only too common after operations about the mouth, and generally due to the *streptococcus pyogenes*. In pyæmic conditions pneumonic complications are frequent, and are produced usually by emboli conveying the causative organism, which may be one of the pyogenic cocci, most commonly the *streptococcus pyogenes* or the *staphylococcus pyogenes aureus*, or the colon bacillus. Lastly, we have the acute croupous or lobar pneumonia, which in many of its characters resembles an acute specific infection.

Thus the sudden onset with rigors and high temperature, the occurrence of the general symptoms before, and their independence of, the extent of the local lesion, the more or less characteristic course, and the undoubted instances of its occurrence after infection and in epidemics, point strongly to some microbial origin.

Friedländer in 1882-83 described an oval encapsuled micro-organism in cases of pneumonia, which he found experimentally caused hepatitis of the lung in mice and guinea-pigs.

In 1883-85 Talamon, Klein, and Sternberg described in pneumonic sputum an oval organism, like Friedländer's encapsuled, which induced pneumonia in animals, and termed by the former *micrococcus lanceolatus*, and by Sternberg *micrococcus pasteurii*, and, although there were certain discrepancies, they believed it to be identical with Friedländer's organism. Subsequently, however, Sternberg recognised that these discrepancies were due to the fact that they were dealing with different organisms, a conclusion that was subsequently confirmed by the researches of Fränkel and Weichselbaum. Fränkel isolated from the rusty sputum of pneumonia an organism which he termed the micrococcus of sputum septicæmia, which was not the same as, and was more constantly present in pneumonia than, Friedländer's organism, and which he found was identical with Talamon and Sternberg's organism. Weichselbaum, in an extended research, obtained Fränkel's organism, which he termed the *diplococcus pneumoniæ*, in a large proportion of his cases, and in only a few that of Friedländer.

There seems to be little doubt that the majority of cases of acute croupous pneumonia are dependent upon this *diplococcus pneumoniæ*, and that Friedländer's organism, now termed Friedländer's pneumococcus, or, better, pneumo-bacillus, is of ætiological significance in only a small minority, if at all. It is, however, associated with certain pathological processes which will be referred to below.

THE DIPLOCOCCUS PNEUMONIÆ.

Synonyms, Fränkel's pneumococcus, *micrococcus pasteurii* (Sternberg), *micrococcus lanceolatus* (Talamon).

This organism is found in large numbers in the rusty sputum and also in the hepatised lung in cases of pneumonia; it occurs usually as an oval coccus united in pairs or occasionally in chains of three or four elements, when it is often almost

spherical, and surrounded generally by a well-marked capsule (fig. 57). It is non-motile, stains with the ordinary anilin dyes, and by Gram's method. It is aerobic and also facultative anaerobic, but is by no means easily cultivated. On glycerin agar and on serum at 37° C. it forms minute, transparent, almost invisible colonies like droplets of fluid. It does not grow on gelatin at the ordinary temperature, but in a 120 per cent. gelatin at 25° C. minute white colonies develop without liquefaction. In broth it produces a faint cloudiness ;

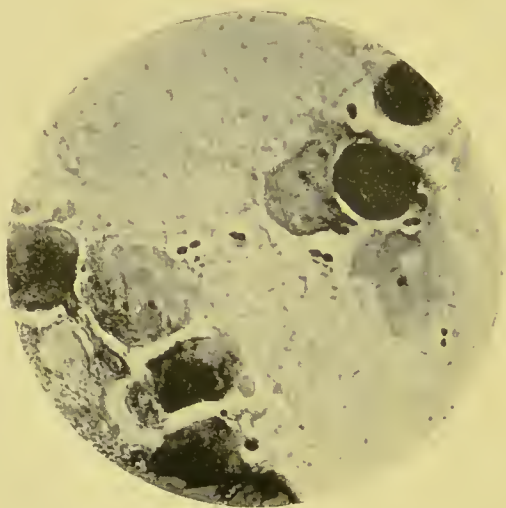


FIG. 57.—FRÄNKEL'S PNEUMOCOCCUS. COVER-GLASS PREPARATION OF BLOOD.
× 1000.

it does not grow on potato, but develops in milk, which is coagulated ; neutral litmus sugar-agar becomes red during its growth, showing the production of acid. On the ordinary culture media it retains its vitality for a short time only, not more than about a week ; but if a little blood be smeared over the surface of the agar it retains its vitality for a month or even longer. Foa's method for keeping Fränkel's pneumococcus alive and virulent is to receive the infected blood into small glass tubes 5 mm. in diameter and 20 cm. long, so that the

blood completely fills the tube, which is then sealed and kept away from the light at the ordinary temperature.¹

Under cultivation it usually assumes the form of a streptococcus (fig. 58) and the capsule is lost, but is regained again on passage through a susceptible animal. A good deal of variation occurs in the morphology of the organism obtained from different sources and under cultivation. Its thermal death-point according to Sternberg is 52° C., the time of exposure being ten minutes, and it is readily destroyed by the ordinary

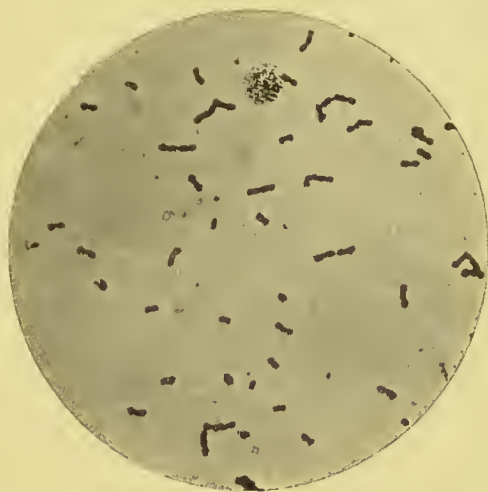


FIG. 58.—FRÄNKEL'S PNEUMOCOCCUS. COVER-GLASS PREPARATION FROM A GLYCERIN-AGAR CULTURE 48 HOURS OLD. $\times 1000$.

germicides. In order to isolate it several tubes of glycerin agar may be inoculated with rusty sputum and incubated for forty-eight hours; in some a pure culture may be obtained. A far more certain method is to inject a drop or two of the rusty sputum into the peritoneal cavity of a mouse or guinea-pig. The animal will die in from twenty-four to thirty-six hours, and the organism will be found in considerable numbers in the lung and blood, from which cultures may

¹ *Brit. Med. Journ. Epit.* 1896, I. p. 68, No. 324.

be obtained. It varies considerably in virulence, and under cultivation this may be completely lost, while the virulence may be increased by a series of passages through a susceptible animal. The less virulent it is the longer it tends to retain its vitality under cultivation.

The organism is pathogenic for mice, rabbits, and guinea-pigs, and death follows from subcutaneous, intravenous, and intraperitoneal or intrathoracic injection of a virulent culture, or of rusty pneumonic sputum, in twenty-four to forty-eight hours. Mice and rabbits are the most susceptible animals, while pigeons and fowls are immune. Except when injected into the lung or into the trachea, pneumonia does not result, but the disease runs the course of a septicæmia with high temperature and dyspnœa, death being generally preceded by a subnormal temperature and often convulsions. The post-mortem appearances are much œdema and inflammatory infiltration at the seat of inoculation, hæmorrhages in the serous membranes, enlargement and congestion of the spleen, and congestion of the lungs. The organisms occur in large numbers in the blood, lungs, and spleen, usually in the form of oval diplococci with well-marked capsules, but sometimes as short chains of streptococci. When injected into the lung or trachea a typical fibrinous or croupous pneumonia results.

Immunity can be conferred on susceptible animals by treating them with attenuated cultures, and also by inoculation with increasing doses of filtered broth cultures of the virulent organism. G. and F. Klemperer used recent broth cultures heated to 60° C. for one or two hours. Washbourn¹ has used filtered cultures in defibrinated blood, 20 c.c. of which injected subcutaneously in a rabbit conferred immunity against virulent cultures, an immunity persisting for fifty or sixty days. The blood serum of such immunised animals will protect other animals when injected, and Klemperer, Issaef, and Washbourn have prepared a pneumonic antitoxin.

¹ *Trans. Path. Soc. Lond.* xlv. 1895, p. 315.

The latter by first immunising a horse with filtered cultures increases the immunity by injection with gradually increasing doses of living virulent cultures, until a very high degree of immunity is obtained; the blood serum may then be used as an antitoxin. The protective serum seems to produce aggregation of the cocci when added to a culture of the diplococcus. Klemperer and Washbourn have found that the serum of convalescent patients possesses some degree of protective power. The serum, however, taken during the pyrexial stage of the disease rather increases the susceptibility of animals to pneumococcic infection.

As regards the nature of the pneumonic toxins, Auld¹ reports that he has separated a proteose and an organic acid from the blood and organs of infected animals and from cultivations of the *diplococcus pneumoniae* in alkali-albumin; apparently the same products were obtained, the alkaline medium soon becoming permanently acid. The proteose on subcutaneous or intravenous injection produced some fever, on intrathoracic injection fever and dyspnoea, and post-mortem pleurisy and consolidation of the lung were found. The organic acid produced slight rise of temperature, but nothing else.

The production of a typical pneumonic process experimentally and the presence of the diplococcus in a large proportion of cases of acute croupous pneumonia point to its specific relationship to the disease. With regard to the latter observation, Weichselbaum obtained it in ninety-four cases out of 129 examined, Wolf in sixty-six out of seventy cases, and Netter in 75 per cent. of the cases examined.

The organism is frequently present in the saliva of healthy individuals, as shown by Netter, Sternberg, and others, and the generally accepted idea of the relation of 'catching cold' to an attack of the disease is explicable on the theory that the action of cold lowers vitality, and renders the tissues

¹ *Brit. Med. Journ.* 1897, i. p. 775.

vulnerable to the attacks of the organism already in close relation to them.

In addition to acute croupous pneumonia, the *diplococcus pneumoniae* is associated with a number of other important pathological conditions in man. It is a pyogenic organism, producing abscesses when inoculated into an insusceptible animal such as a dog, and has been met with in abscesses, empyema, and purulent arthritis. It is also found in about half the cases of purulent meningitis, sometimes in cerebro-spinal meningitis, in about a third of the cases of otitis media and ulcerative endocarditis, and occasionally in peritonitis.

FRIEDLÄNDER'S PNEUMO-BACILLUS.

This organism, already referred to above in the general discussion of pneumonia, and originally believed by Friedländer to be the cause of the disease, has only been obtained by recent observers in a small proportion of cases. It is a very pleomorphic organism, occurring in sputum or in the blood of an inoculated animal generally as a short rod with rounded ends surrounded by a marked capsule. In cultivations it forms short rods, long rods, chains, and even filaments, the capsule being absent, but this is regained on passage through a susceptible animal. It is aerobic and facultative anaerobic, non-motile, does not form spores, does not produce indol, and is readily stained with the ordinary anilin dyes, but not by Gram's method, an important distinction from the *diplococcus pneumoniae*. It grows readily on the various culture media from 20° to 37° C., on agar and blood serum forming a copious, viscid, greyish growth, on gelatin a thick, white, shining, porcelain-like growth without liquefaction; and in stab-cultures in gelatin, a so-called nail-shaped growth is developed (fig. 59), consisting of a white growth along the needle track, tapering from above downwards, and at the surface heaped up and

expanded, forming the 'head' of the nail. On potato a copious whitish growth develops, while milk is curdled and gas bubbles frequently form in stab-cultures.

Grimbert has examined the fermentations excited by the *bacillus pneumonicæ* of Friedländer, and finds that it ferments not only glucose, but galactose, arabinose, mannite, saccharose, maltose, lactose, raffinose, dextrin and starch, glycerin and dulcitol. The fermentation products were ethyl alcohol, acetic acid, lactic acid (lævo-rotatory) and succinic acid. Mannite gave lactic acid (lævo-rotatory) only, while its isomer dulcitol produced succinic acid.

The pneumo-bacillus of Friedländer is pathogenic to mice and guinea-pigs, but rabbits are immune. Post-mortem, the spleen is enlarged and the lungs congested with patches of consolidation, the organism being found in large numbers in the blood. It is possible that this organism may occasionally set up a pneumonic or bronchitic process in man, but this is doubtful. It seems to be occasionally associated with anginal conditions, which are characterised by the formation of a false membrane with an absence of any general symptoms.¹ A microscopical examination of the membrane will show the organism surrounded with the capsule and unstainable by Gram's method. If a culture be made on serum, the large, round, greyish colonies of the bacillus will be recognisable in fifteen to twenty hours, and should be examined microscopically. To obtain a pure culture a white mouse should be inoculated from a colony :

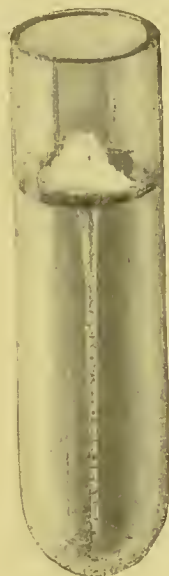


FIG. 59.—FRIEDLÄNDER'S PNEUMO-BACILLUS. GELATIN STAB-CULTURE, 7 DAYS OLD.

¹ *Ann. de l'Inst. Pasteur*, xi. 1897, p. 67 (Nicolle & Hébert). See also Pakes, *Brit. Med. Journ.* 1897, i. p. 715.

it will die in twenty-eight to sixty hours. Friedländer's pneumo-bacillus has also been met with in water by Grimbert.¹ According to him it is identical with the *B. capsulatus* of Mori.

CLINICAL EXAMINATION (PNEUMONIA).

1. Make cover-glass specimens from the rusty sputum and stain some with Löffler's blue, and others by Gram's method with eosin. By a microscopical examination the oval diplococci will be readily recognised, Friedländer's pneumo-bacillus being distinguished from the *diplococcus pneumoniae* by decolourising by Gram's method. The latter organism is the only one, however, which is likely to be ordinarily met with.

2. If the diplococci are found to be pretty abundant in the sputum, and other organisms nearly absent, an attempt may be made to cultivate by inoculating several glycerin-agar tubes and incubating at 37° C. for forty-eight hours.

3. If the diplococci are scanty, or so mixed with other organisms that it is difficult to be sure of them and probably impossible to obtain a pure culture, a drop or two of the sputum should be injected into the peritoneal cavity of a mouse or guinea-pig. The animal will die in from twenty-four to thirty-six hours, and the *diplococcus pneumoniae* will be found plentifully in cover-glass specimens prepared from the blood or lung-juice, and pure cultures can be readily obtained by inoculating glycerin-agar tubes with the blood or lung-juice.

4. The culture or inoculation method, preferably both, will probably have to be adopted for the recognition and isolation of the *diplococcus pneumoniae* in pus from empyemata, abscesses, &c.

5. Friedländer's pneumo-bacillus can be readily isolated by making gelatin plate cultivations, in which its colonies form white, shining, heaped-up points.

INFLUENZA.

A minute bacillus has been described in this disease by Pfeiffer and Klein. Pfeiffer in 1892 was the first to report its discovery; he found it in large numbers in the bronchial

¹ *Ann. de l'Inst. Pasteur*, x. 1896, p. 708.

secretion. The influenza bacillus is perhaps the smallest bacillus with which we are acquainted. It is a minute rod about the same diameter, but only half as long, as the bacillus of mouse septicaemia. It does not stain by Gram's method, and not very readily with the ordinary dyes; dilute carbol-fuchsin or prolonged staining with Löffler's blue yield the best results, the poles tending to stain more deeply than the centre. In the sputum it occurs singly, in small groups, or in larger masses, being most numerous at about the height of the disease. In order to isolate the organism a patient with bronchial expectoration should be chosen; he rinses his mouth and gargles his throat with hot water several times, and then, after coughing, the expectoration is received into a little sterile normal salt solution. A little of this expectoration is now washed about three times by placing in a test-tube and shaking with sterile salt solution, then repeating the washing in a second, and finally in a third test-tube, with sterile salt solution. By means of a platinum needle a number of agar and broth tubes are inoculated with the sputum after the last washing, and incubated at 37° C. The bacillus is aërobic and non-motile, and spores have not been observed. No growth occurs on media at 22° C. On glycerin agar and blood serum at 37° C. it forms very small, transparent, drop-like colonies in from twenty-four to forty-eight hours, which, according to Kitasato, never become confluent. In broth it grows at the surface in fine white flakes, which subsequently sink.

In an agar stab a delicate white growth occurs along the line of puncture with minute pear or club-shaped projections from it. There is no growth on potato.

It soon dies out in cultivation, but according to Klein can be kept alive for some weeks in gelatin incubated at 37° C. The liquefied gelatin remains clear, and a fine flocculent growth forms a precipitate at the bottom. Cover-glass preparations from cultures show long twisted chains and

threads of bacilli, aggregated so as to form dense networks and convolutions. These chains or threads are composed of bacilli placed end to end, and united by a continuation of the cell-membrane. Involution forms occur.

Canon states that he obtained this bacillus in the blood in a number of cases, but many other investigators have failed to find it. Klein obtained it in six cases out of forty-three. According to Pfeiffer the bacillus is only pathogenic to monkeys and rabbits. Klein, however, was unable to obtain any definite effects in these animals by the injection either of sputum rich in bacilli or of pure cultures.

The influenza bacillus is met with in all uncomplicated cases of influenza in the bronchial secretion, often almost in pure culture, and in the bronchial tubes and lung in the pneumonic complications accompanying the disease. The organisms disappear with convalescence, and are not met with in other diseases. Klein¹ appears to consider that the pneumonia often accompanying the disease is probably directly due to the bacillus.

CLINICAL EXAMINATION.

In cases of influenza, accompanied with bronchitis or pneumonia, the influenza bacillus may be met with in large numbers almost in pure culture, and their presence may aid in confirming the diagnosis. Cover-glass preparations may be stained with carbol-methylene-blue.

¹ 'Further Report on Epidemic Influenza, 1889-92,' *Local Gov. Board Report*, 1893, p. 85.

CHAPTER XIV.

ACTINOMYCOSIS—MYCETOMA—LEPTOTHRIX BUCCALIS—CLADOTHRIX DICHOTOMA—MYCOSIS TONSILLARIS.

ACTINOMYCOSIS.

It is only comparatively recently that actinomycosis has been recognised as a disease affecting man. In its clinical history and pathological lesions it closely resembles tuberculosis, and doubtless cases of it have been, and are, frequently confounded with that disease.

In cattle, actinomycosis has long been known, but its exact pathology was involved in considerable doubt until the researches of Bollinger in 1876. It affects chiefly the tongue, jaw, face, and throat, and was described under such varied names as wen, scrofula, scirrhus, osteo-sarcoma, cancer, wooden tongue, &c. The following is a description of a typical case in a cow from 'The Veterinarian' for 1841 :—

'The animal was a three-year old heifer. About five or six weeks ago the owner observed that she was losing flesh, that the saliva was dripping from her mouth, and that her cheeks appeared enlarged. A few days afterwards he examined her mouth and found a considerable portion of masticated food impacted between the molar teeth and the muscles of the cheek. This he removed, but a few days afterwards he observed that there was great difficulty in ruminating. He then examined her teeth, but could not detect anything amiss with them. A week after a veterinary was called in. This gentleman found her standing up, trying to chew the

cud; she evinced much tenderness of the mouth, the saliva was flowing profusely, and the tongue slightly protruded; she was very much emaciated, and her milk nearly gone. Upon examination her tongue was found enlarged, appearing shorter than natural, and hot and very tender. There was considerable enlargement in the submaxillary space, the tongue almost of a natural colour. She was very eager for food, but could not manage to get much of it over the tongue.'

Shortly afterwards the animal was killed, and the veterinary who made the post-mortem reported as follows:—

'Animal very much emaciated. Tongue enlarged from near the root almost to the tip, but very little altered in colour. Upon cutting into it great resistance was offered to the knife, the cut surface feeling hard and granular. In some parts there were little abscesses, containing thick yellowish matter, of the size of a horse-bean. The whole substance appeared studded with tubercles. The disease extended throughout the whole of the tongue. I believe the disease may be termed "scirrhus," or what is vulgarly called cancer of the tongue.'

On cutting into a 'wooden tongue,' or wen, a grating sensation is felt, such as that experienced in cutting a turnip or unripe pear; on examining the section little rounded, yellowish, frequently almost caseating, areas will be noticed, resembling old tubercles. On making sections and examining with a low power it is found that these rounded areas are composed of masses of small lymphoid cells, with occasionally giant cells, surrounded by a capsule of fibrous tissue. The growth may be so soft that it is practically purulent, and abscesses varying in size from a pin's head to that of an orange may be present in the diseased areas. Like tubercles, the growths may become caseous, calcified, or fibrous. In the growth or in the pus from abscesses, when examined fresh with a low power, yellowish or yellowish-white granules will be found here and

there, which may be very minute or as large as a small pin's head, and are somewhat soft in consistence and on slight pressure flatten out. Examining with a higher power, these granules are found to contain round, ovoid, or reniform bodies which have a rosette-like appearance, a more or less structureless centre with club-shaped bodies radially arranged around the periphery (fig. 60). These peculiar structures are the cause of the disease, and are the form assumed in the animal body by an organism belonging to the streptothrix group

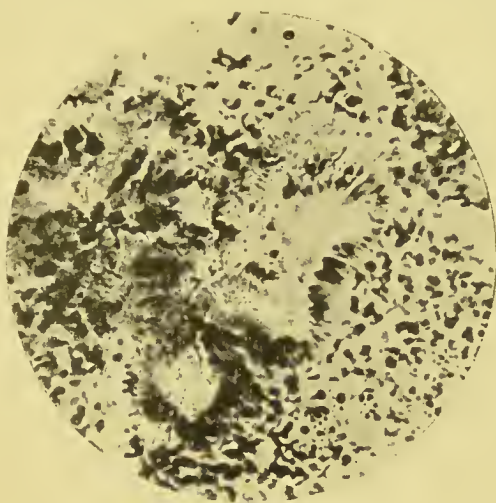


FIG. 60.—ACTINOMYCOSIS BOVIS. TONGUE. GRAM. $\times 350$.

termed the actinomyces, or, from its appearance, the ray fungus.

Sections of the diseased tissues show the structure of the organism still better. Gram's method usually gives good results, and it will generally be found that the following appearances can be observed: Surrounded by the lymphoid cells are the reniform or ovoid bodies, situated at the periphery of which are radially arranged, club-shaped structures deeply stained with the gentian violet, while the central portion is unstained and structureless, or contains granular

matter or calcareous particles. Various appearances may be met with in different parts of the section, according as the actinomycotic nodules are cut through their centre or periphery ; when the latter is the case, the clubs are shown in transverse section and appear as closely packed, deeply stained dots. Sometimes, however, in addition to the clubs the centre of the rosette is occupied by numerous interlacing filaments, also stained by the gentian violet.

In man, actinomycosis is often associated with suppuration. If a little of the pus be examined it will pro-

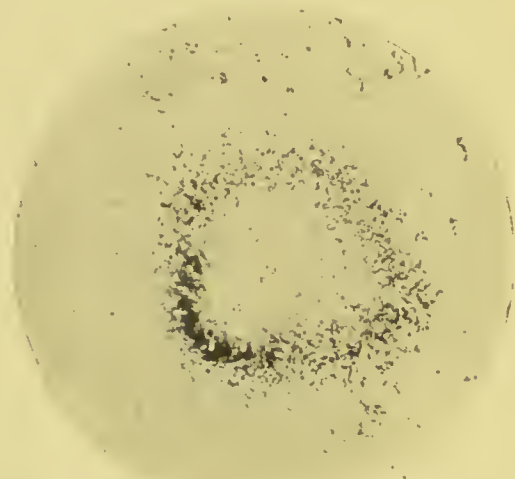


FIG. 61.--ACTINOMYCOSIS HOMINIS. LIVER. GRAM. $\times 350$.

bably contain tiny yellowish or sulphur-yellow granules, which, microscopically, are found to have the same structure as the granules met with in the disease in cattle and described above—namely, rosette-like tufts of clubs, but in addition, occupying the centre of the tufts, is a mass of fine tangled filaments, the ends of which are frequently seen to be continuous with the clubs. In teased-up specimens, or in sections stained by Gram's method, an appearance is observed very different from that of the bovine variety, viz. tufts of

interlacing filaments stained by the gentian violet, but a complete absence of purple clubs (fig. 61). The clubs, however, are not really absent; they are present abundantly all round the periphery of the filamentous tufts, in a stunted condition it is true, and they do not stain by Gram's method. These clubs are very well seen in fresh specimens of the pus or in unstained sections, or by staining with orange-rubin, or the Ehrlich-Biondi reagent (fig. 62). The conditions in cattle and man, at first sight so very different, are thus seen to be similar, and

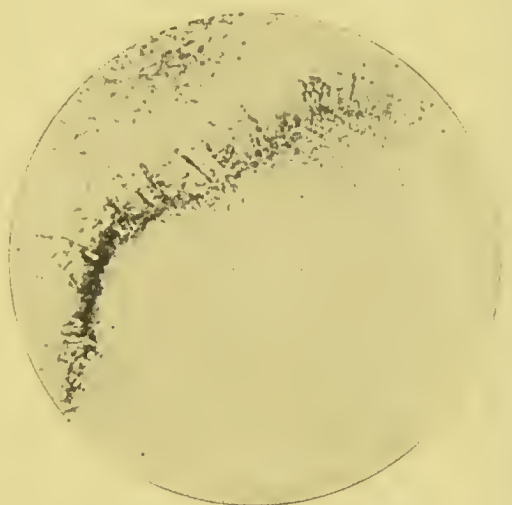


FIG. 62.—ACTINOMYCOSIS HOMINIS. SAME TISSUE AS FIG. 61. STAINED WITH EHRLICH-BIONDI STAIN AND SHOWING CLUBS. $\times 750$.]

their identity is further established by the occasional occurrence in cattle of filamentous tufts, staining by Gram's method, within the rosettes, and by the clubs in man now and then taking on the gentian violet stain.

The cultivation of the actinomyces can be performed by collecting the pus from a case of the disease in sterilised tubes, and subsequently turning it out into a sterilised capsule and picking out the actinomycotic granules with sterilised needles, planting these on the surface of glycerin agar, and in-

cubating at 37° C. A certain number of the tubes will probably be uncontaminated, but in others a growth of the *staphylococcus pyogenes aureus* or other pyogenic organism, which is not unfrequently associated with the actinomyces, may occur. In the uncontaminated tubes, a growth begins to appear in a few days in the form of little colonies of a tough membranous consistence, somewhat crinkled, greyish,



FIG. 63.—ACTINO-
MYCES. POTATO
CULTURE, 3
MONTHS OLD.

and shining, while the agar beneath them becomes stained brownish. The growth increases and the colonies coalesce, forming a brownish, crinkled, membranous expansion, sticking firmly to the agar and difficult to remove or break up, while the agar becomes stained brown throughout; later on the membranous growth may become dappled with yellow as though powdered with flowers of sulphur, or occasionally whitish. In gelatin, little spherical, feathery tufts develop and sink to the bottom as the gelatin is liquefied. On potato a remarkable growth develops; at first brownish, it afterwards becomes almost black and is very thick or heaped up with a much-wrinkled surface, while later on it has the appearance of being sprinkled with flowers of sulphur (fig. 63). In broth it forms delicate feathery flocculi.

Cover-glass specimens from young agar cultures show masses of tangled filaments, which appear to be more or less branched, and stain well with the ordinary anilin dyes and by Gram's method; with the latter the filaments often appear more or less beaded, but no trace of rosette formation or even of clubs is to be found in the cultures (fig. 64). Various views have been held as to the botanical position of the actinomyces. It was considered by Crookshank and others to be a fungus belonging to

the basidiomycetes, the club-shaped structures being regarded as spore-bearing organs or conidia ; now, however, it is generally believed to be a streptothrix, though Sauvageau and Radais would include it among the bacteria under the name *oospora bovis*. In the pus the filaments can sometimes be seen, especially if stained by Gram's method with orange rubin (Crookshank). Inoculated into the peritoneal cavity of rabbits and guinea-pigs the cultivated organism reproduces the disease, numerous actinomycotic nodules forming in the

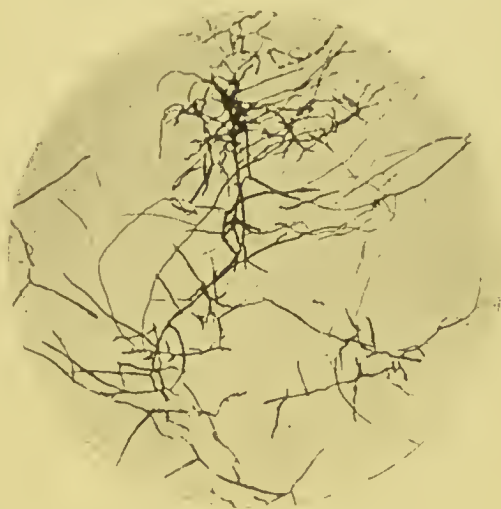


FIG. 64.—ACTINOMYCES. COVER-GLASS PREPARATION.
GRAM. \times 750. ~

peritoneum and elsewhere. There is much doubt as to the mode of spread of the disease, and the infection of man by it. It does not seem to be particularly contagious, and diseased and healthy animals are often placed together without bad result ; it can, however, be conveyed by direct inoculation, as shown by the experiments of Jone and Ponfick, calves inoculated intraperitoneally with portions of diseased tissues dying after some weeks or months, with an abundant development of actinomycotic nodules. Crookshank has also infected

a calf with the material from a human case. Feeding experiments give negative results. The general view now held is that the organism occurs on cereals, straw, or roots, and gains access to the system through slight scratches or wounds in the mucous membrane of the mouth, pharynx or larynx. In man no source of infection can be traced, though one or two cases are reported where the disease has occurred after eating grains of barley. The disease is met with not only in cattle, but also in horses and swine. In the latter case there may be considerable calcification in the nodules, and it may be necessary to decalcify with dilute nitric or hydrochloric acid before the rosettes can be stained.

It is important to note that tuberculin may cause a reaction in actinomycosis, similar to that which occurs in tuberculosis, and as the actinomycotic lesions are very like those which are found in the latter disease, mistakes may easily be made, and can only be avoided by a microscopical examination. It is of considerable practical importance to distinguish actinomycosis from tuberculosis, for in the majority of cases of the former, both in man and animals, iodide of potassium exerts a specific curative action.

CLINICAL EXAMINATION.

1. Pour out the pus or discharge into a large capsule or Petri dish so that it forms a thin layer, look for any yellowish or other granules, pick them out with a needle, and place on a clean slide in a drop of 50 per cent. glycerin. If no granules can be found, a little of the discharge may be spread on a slide with a drop of 50 per cent. glycerin. Cover with a cover-glass and apply a little pressure. Examine with a $\frac{2}{3}$ -inch objective. If any actinomycotic tufts are present they will be seen as yellowish or pale brownish, spheroidal, ovoid, or reniform masses, and with a $\frac{1}{6}$ -inch objective these will be found to have a radiating structure from the presence of the clubs.

2. Stain cover-glass specimens of the discharge, by Gram's

method, with cosin. The actinomycotic tufts in the majority of instances will be found to consist of little masses of tangled filaments stained blue and surrounded by a pink zone which has an indistinct radiating structure.

N.B.—In the majority of cases of *actinomycosis hominis* the clubs do not stain by Gram's method. The reverse is the case with *actinomycosis bovis*.

3. Sections of actinomycotic tissue are best prepared by the paraffin method. If frozen, the actinomycotic nodules are very apt to fall out. Sections may be stained in the following ways :—

(a) By Gram's method, with eosin.

(b) With the Ehrlich-Biondi triple stain. Stain for from half an hour to two hours. Place in methylated spirit until sections appear greenish, then pass through absolute alcohol and xylol. The clubs are stained yellowish-brown, and are well shown in human cases when unstained by Gram's method.

(c) By Plaut's method. Stain in warm carbol-fuchsin for ten minutes, rinse well in water, stain in a saturated solution of picric acid in methylated spirit for five to ten minutes, rinse well in water, place in 50 per cent. alcohol for ten minutes, pass through absolute alcohol and xylol.

(d) Good preparations are also obtained by staining in Ehrlich's hæmatoxylin and counter-staining with orange-rubin. This also shows the clubs when they are unstained by Gram's method.

MADURA DISEASE OR MYCETOMA.¹

The madura disease, otherwise known as madura foot, mycetoma, or the 'fungus disease,' is a chronic local affection generally attacking the foot, occasionally the hand, sometimes extending up the leg, but rarely to the trunk. The disease occurs in certain districts in India, and full descriptions of it have been given by Vandyke Carter and Lewis and Cunningham. A 'madura' foot appears enlarged, and numerous

¹ For bibliography see a paper by the writer, *Trans. Path. Soc. Lond.* 1893.

sinuses with raised mammilated apertures open on the surface. On making a section into the diseased tissues the bones are found to be more or less carious, while the soft structures are tough and hypertrophied from the occurrence of chronic inflammatory changes. Numerous small cavities are present, sometimes filled by yellowish particles resembling fish-roe, and hence termed 'roe-like particles'; at other times, however, containing black particles of irregular shape, coal-like consistence, and variable size, exceptionally as large as a marble or walnut. The presence of the white or black granules, which may be discharged from the sinuses before mentioned, divides the disease into two classes—the so-called white and black varieties. Lewis and Cunningham have also described a third variety in which the granules are red like cayenne pepper.

Vandyke Carter¹ first drew attention to the similarity of the white variety in its microscopical characters to actinomycosis. In sections stained by Gram's method, more or less crescentic or reniform bodies are noticeable, divided into wedge-shaped areas, which contain masses of fine filaments stained purple. Surrounding the crescentic bodies is a zone of radially arranged elements, many of which are fan-shaped owing to branching; they are indistinct, as they do not stain with the gentian violet, but they are very suggestive of the club-shaped structures present in actinomycosis, and they resemble the *actinomycosis hominis* inasmuch as they do not stain by Gram's method (fig. 65). By staining with hæmatoxylin and orange-rubin, or with the Ehrlich-Biondi triple stain, here and there in the radial zone well-defined clubs can be demonstrated. It is therefore pretty certain that the radial zone is composed of degenerate club-shaped structures, and the disease evidently closely resembles actinomycosis, and may be a variety of it.

¹ *Bombay Med. and Phys. Soc.* ix. 1886 (new series), p. 86.

Boyce¹ cultivated from a case of the white variety a streptothrix which differed somewhat from the actinomyces; it grew slower, and produced no pigment. On agar it formed white, raised colonies with radial grooves, not unlike the tiny barnacles found on wooden piles in the sea. Vincent² also isolated a streptothrix perhaps identical with that of Boyce. He describes it as forming on glycerin agar umbilicated colonies, first white and afterwards red. It differed from the actinomyces in growing feebly in broth, in not liquefying gelatin, and in not being inoculable in the rabbit.

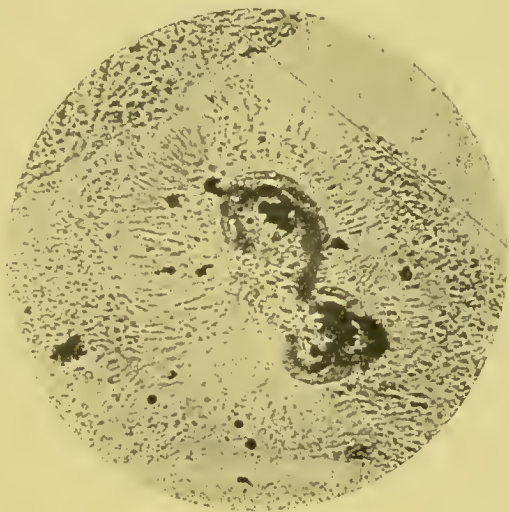


FIG. 65.—MADURA DISEASE. WHITE VARIETY. GRAM AND EOSIN. $\times 350$.

The relation of the black to the white variety of madura disease has been somewhat debated. Kanthack³ described the black variety as being probably a late stage of the white. It seems, however, that the co-existence of the two conditions in the same specimen is very rare, and Boyce and Surveyor,⁴ after a critical examination of a

¹ *Hygienische Rundschau*, 1894, No. 12.

² *Ann. de l'Inst. Pasteur*, 1893.

³ *Journ. Path. and Bact.* 1892.

⁴ *Proc. Roy. Soc. Lond.* 1893, and *Phil. Trans. Roy. Soc. Lond.*

large number of specimens, came to the conclusion that the black variety was a distinct disease, and due to an organism belonging to the group of the higher fungi, the black particles or masses being the lignified mycelium or sclerotium such as is met with in ergot. Paltauf¹ would also ascribe the white variety to one of the higher fungi, but most authorities do not hold this view. Le Dantec² met with a case of the black variety in Senegal from which he cultivated a small bacillus, but this is not unlikely to be a disease distinct from mycetoma. . . The writer (*loc. cit.*), in an examination of three specimens of madura disease, met with one which could not be classed with either the white or the black variety, and in which structures suggestive of a sclerotium were present.

It would seem that there are probably several conditions, both in actinomycosis and in mycetoma, having a general resemblance but differing slightly, and dependent upon varieties of a streptothrix.

Gasperini³ considers that there are several species (or varieties) of the actinomyces organism, viz. *A. bovis*, *albus*, *sulphureus*, and *luteo-rosens* in the ox, and *A. albus*, *asteroides* and *carneus* in man, while madura disease he regards as a form of actinomycosis due to the *A. ruber* and other species.

MYCOSIS TONSILLARIS (MYCOSIS PHARYNGIS LEPTOTHRICIA).

A chronic disease attacking young adults, resistant to treatment, and characterised by the presence of small, white, tough adherent excrescences on the mucous membrane of the pharynx. Microscopically, the patches consist of collections of epithelial cells and débris, infiltrated with leptothrix filaments and bacteria. The disease, however, seems to be a keratosis, infection with the organisms being secondary.

¹ *Semaine médicale*, 1894, p. 298.

² *Arch. de méd. navale*, Dec. 1894.

³ *Atti Soc. Tosc. Sci. Nat.* x. 1896, p. 144.

But occasionally a true 'mycosis' apparently occurs, readily amenable to treatment and due to a leptothrix.¹

LEPTOTHRIX BUCCALIS.

Long threads of considerable size, compared with the filamentous forms of even large bacteria, often occurring in large numbers in the mouth. They become of a violet colour if treated with iodine and then with a dilute acid. It is stated to be an important factor in the production of dental caries, gaining access through cracks or flaws in the enamel, and causing destruction of the dentine.

CLADOTHRIX DICHOTOMA.

An organism not unfrequently met with in natural waters. It forms long threads, straight, or sometimes slightly undulating, or even spiral and apparently branched, though the branching is not a true one. It can be cultivated on the ordinary laboratory media at room temperature, forming on agar a brownish, wrinkled, tough, membranous layer, very adherent, and staining the medium beneath it a pale brown, not unlike the actinomyces in these respects. It is non-pathogenic.

¹ See *Glasgow Medical Journal*, No. 2, August, 1896, p. 81 *et seq.* (Brown Kelly).

CHAPTER XV.

TETANUS—MALIGNANT ŒDEMA—SYMPTOMATIC ANTHRAX—
CLOSTRIDIUM BUTYRICUM.

TETANUS.

THE causation of tetanus was for a long time involved in mystery. No obvious or characteristic changes being met with after death, the disease was regarded by many as 'functional,' a convenient term which commits us in no way and expresses little. Others believed that a primary lesion of the central nervous system might be the cause of the affection, while a few classed it with the specific diseases. Researches made during the last fifteen years have shown that tetanus is an acute infective disease, the specific organism of which has been isolated and its chemical products investigated.

It had long been noticed that wounds soiled with earth were specially prone to be followed by tetanus, and Sternberg in 1880, and Nicolaier in 1884, produced tetanus in rabbits by introducing a little garden earth beneath the skin. The latter observer found at the seat of inoculation and in his impure cultures—for he was unable to obtain pure ones—a distinctive bacillus, and he was able with these cultures, and with the pus from the seat of inoculation, to induce tetanus in other animals. Carle and Rattone subsequently showed that the bacillus of Nicolaier was present in the tissues of, and secretions from, the wound, in cases of traumatic tetanus in man, and that inoculation with the pus from

such a wound produced tetanus in the lower animals, observations which were confirmed by Rosenbach in 1885.

The *bacillus tetani* is a straight, slender rod with rounded ends, but under cultivation the rods may grow into longish filaments. It is somewhat motile and possesses a large number of flagella, three or four of which are generally thicker than the rest.¹ Spores are freely formed; they are spherical and develop at the extremity of the rod, and their diameter being much greater than that of the rod, the spore-bearing

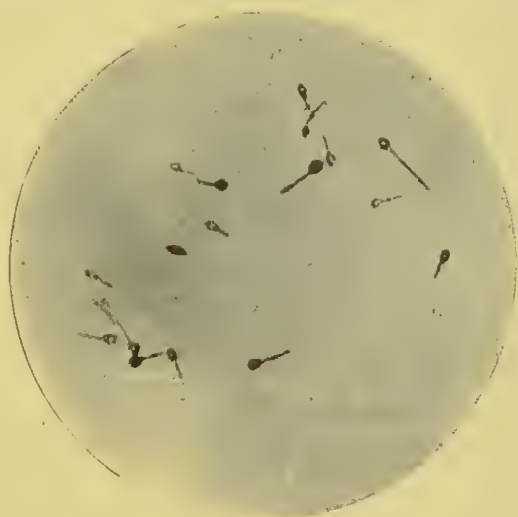


FIG. 66.—TETANUS BACILLUS. COVER-GLASS PREPARATION. $\times 1000$.

organism has been likened to a 'pin' or 'drum-stick' (fig. 66). It stains with the ordinary anilin dyes, and also by Gram's method.

It is a strictly anaërobic organism and will not grow in the presence of a trace of oxygen, nor in an atmosphere of carbon dioxide. It can be cultivated in deep stabs in sugar-agar and gelatin, or in broth, by growing in an atmosphere of nitrogen in Buchner's tubes, or in one of hydrogen by the

¹ Kanthack and Connell, *Journ. Path. and Bact.* iv. 1897, p. 452.

writer's method (p. 56). In a gelatin stab-culture at 22° C. the growth radiates from the central puncture, and the gelatin is slowly liquefied. In a stab sugar-agar, it forms a feathery, radiating, outgrowth from the central puncture, a small amount of gas being formed (fig. 67). Broth becomes turbid with the formation of some gas and the development of a foul odour. There is no film formation. The colonies have a central opaque portion surrounded by diverging rays.

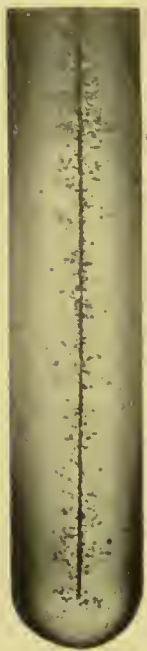


FIG. 67. — TETANUS BACILLUS. STAB-CULTURE IN SUGAR-AGAR, 7 DAYS OLD.

The isolation of the tetanus bacillus is a difficult matter. Chantemesse and Widal, by an elaborate method, obtained pure cultures, which, however, were non-virulent. Kitasato in 1889 obtained it in an ingenious manner: he prepared cultures from the pus of the inoculation wound in a case of tetanus and so obtained a mixed culture just as Nicolaier had done. These impure cultures were examined microscopically at short intervals, and when sporing rods of the tetanus bacillus were observed the cultures were heated to 80° C. for three-quarters of an hour. The effect of the heating was to destroy everything but bacterial spores. By then preparing anaërobic plates pure colonies of the tetanus bacillus were obtained. The tetanus bacillus retains its vitality for months in

cultures, and the spores retain their vitality for months in the dried state, withstand a temperature of 80° C. for an hour, but are killed by boiling for five minutes. Carbolic acid (1-20) does not destroy the spores under about fifteen hours.

Brieger, from impure cultures of the tetanus bacillus, obtained two basic bodies which he termed tetanine and

tetano-toxin, the former producing tetanic symptoms in mice, and the latter tremor, paralysis, and finally convulsions. Brieger has also isolated tetanine from the amputated limb of a tetanic patient. Brieger and Fränkel obtained a tox-albumin from bouillon cultures which induced tetanus in guinea-pigs. Brieger and Cohn subsequently investigated the tetanus poison obtained by precipitating veal-broth cultures with ammonium sulphate added to saturation. The poison formed a flocculent precipitate which floated on the surface; it was purified by redissolving, precipitating the proteid with basic lead acetate, and removing other soluble impurities by dialysis. So prepared, the tetanus poison forms yellow flakes, odourless, and soluble in water, and not giving the Millon and xanthoproteic reactions. Most of the metallic salts do not precipitate it, and it is not carried down by Roux and Yersin's method with calcium phosphate. It contains no phosphorus and only traces of sulphur. Of the most active preparation 0·00000005 gram killed a mouse. Brieger and Boer have recently obtained the tetanus toxin by the same method as that used for the diphtheria toxin (p. 175).

In a case of tetanus examined by Sidney Martin,¹ an albumose, chiefly deuterio-albumose, was extracted from the blood. Injected into an animal, it produced depression of temperature, followed by progressive wasting, but no spasm or paralysis.

Fermi and Pernossi² found agar cultures of tetanus to be the most toxic—more so than gelatin and bouillon. According to their investigations, the tetanus toxin is destroyed in an hour at 55° C. in the presence of water; it is also destroyed after exposure to direct sunlight for from eight to ten hours. The poison is not an enzyme. Man and the horse are most subject to tetanus; cattle and sheep are rarely affected, while

¹ 'Chemical Pathology of Diphtheria, &c.,' Gulstonian Lectures, *Brit. Med. Journ.* 1892, i. p. 756.

² *Centralb. f. Bak.* March, 1894, p. 303.

the fowl, frog, triton, snakes, and tortoise are immune. Mice, rabbits, and guinea-pigs are all very susceptible. The bacillus is present in the superficial layers of the soil in many localities, but not in others, and this accounts for the fact that tetanus is rare in some places and frequent in others. Curiously enough, some of the savage inhabitants of the Solomon Islands have made use of poisoned arrows, the poisonous nature of which is due to tetanus-bearing earth. The arrows are tipped with a viscid fluid, then rubbed in the soil from a mango swamp, and afterwards dried. Individuals wounded with these arrows usually develop tetanus.

The bacillus is localised to the seat of inoculation, or at most is met with in the nearest lymphatic glands, so that the general symptoms are due to the absorption of the chemical products. The organisms associated with the tetanus bacillus in earth are probably of considerable importance in the production of the disease, for it has been shown that if the tetanus bacilli and their spores be carefully washed so as to remove all adherent toxins, they fail to set up tetanus on inoculation, while if the same washed bacilli be injected, together with a little lactic acid, tetanus follows; the explanation being that the bacilli are unable to multiply unless the surrounding tissues are damaged and phagocytosis prevented. The associated organisms in the wound probably effect this, and do not act by producing a condition of anaërobiosis as has been suggested.

If an animal be cautiously injected with tetanus toxin, by commencing the treatment with a weakened toxin, and increasing the dose very gradually, a high degree of immunity is ultimately obtained, and the blood serum acquires marked antitoxic properties. The toxin is obtained by growing the tetanus bacillus in bouillon in an atmosphere of hydrogen for about three weeks, and filtering through porous porcelain. To obtain an active serum, treatment has to be prolonged,

a horse immunised by the writer¹ requiring six months. The antitoxic serum so obtained is by far the most active with which we are acquainted, and is now recognised as the proper remedy to use in cases of tetanus in man. As regards the value of the antitoxic treatment of tetanus, it cannot be said to be so successful as that of diphtheria, and for this reason: in diphtheria there is in a large proportion of the cases a local manifestation to aid diagnosis before any serious absorption of the toxin has taken place, whereas in tetanus the disease is only recognisable by the symptoms



FIG. 68.—GUINEA-PIG INOCULATED WITH A SMALL DOSE OF TETANUS TOXIN, SHOWING PARALYTIC CONDITION OF RIGHT HIND LEG DUE TO SOME SPASM.

induced by such absorption. Nevertheless, it can hardly be doubted that it is our duty to employ the remedy not only in the fully developed disease, but also in certain cases as a prophylactic (see Appendix).

In the guinea-pig and rabbit tetanus toxin induces tonic and not clonic spasm of the muscles. The earliest muscles

¹ *Brit. Med. Journ.* March 2, 1895.

to be affected are those of the dorsal region, and next those of the hinder extremities (fig. 68).

CLINICAL EXAMINATION.

The symptoms of tetanus are usually so obvious that a bacteriological examination is not needed to establish the diagnosis, and unless there be an evident wound it will be difficult, if not impossible, to detect the tetanus bacillus.

1. Prepare several cover-glass specimens of the pus or discharge, and stain by Gram's method. Examine microscopically, looking for the spore-bearing rods or 'drum-sticks.'

2. If 'drum-sticks' be found, an attempt may be made to isolate the bacillus by making anaërobic plate cultivations either direct from the discharge, or after heating it in capillary pipettes to 80° C. for half an hour.

3. Inoculate two guinea-pigs with the discharge. If they die with tetanic symptoms, treat the pus at the seat of inoculation as in 2.

MALIGNANT ŒDEMA.

Malignant œdema is met with in man in connection with wounds soiled with septic matter, compound fractures, contused and lacerated wounds, &c. Usually there is a putrefactive and œdematous condition of the tissues with subcutaneous emphysema. Animals also occasionally suffer from the disease, which can be produced artificially by inoculation with dust, dust from straw, the upper layers of garden earth, and decomposing animal and vegetable matter.

If a guinea-pig be inoculated subcutaneously with a little garden earth it will very likely die in forty-eight hours. Post-mortem, the subcutaneous tissues around the seat of inoculation will be found to be œdematous and blood-stained, with more or less development of gas. The internal organs are only slightly altered, but the spleen may be somewhat enlarged. The juice from the seat of inoculation will be found to

contain a mixture of organisms, but in the blood and organs few will be found. Under the capsule of the spleen, however, long slender rods may be seen: these are the bacilli of malignant œdema.

The bacillus of malignant œdema is a long and slender rod, several of which may be united into a thread. It is motile, and possesses several flagella. It is readily stained by the ordinary anilin dyes, but not by Gram's method.

It spores freely at temperatures above 20° C., the spores being large and central. It is a strictly anaërobic organism, but grows freely when oxygen is excluded. In a deep stab in sugar-agar it forms a thick track of growth, with irregular outline and greyish-white in colour. There is profuse development of gas, accompanied by a foul odour, and attended with rupture of the medium into several portions.

The bacillus of malignant œdema is an organism which has to be distinguished from anthrax, and there should be no difficulty in doing this. Post-mortem, the spleen is rarely much enlarged in malignant œdema, the organism is not very abundant, is almost entirely absent from the blood, is only found under the capsule of the spleen, and not at its centre. If, however, several hours have elapsed since death occurred, the organism may have wandered into the blood and centre of the spleen. The bacillus of malignant œdema is motile, the anthrax bacillus non-motile; the former occurs as a long slender filament, which on staining is seen to consist of two or three long segments; it does not stain by Gram's method, and is strictly anaërobic. Access of oxygen may render the malignant œdema bacillus non-motile.

CLINICAL EXAMINATION.

The character of the wound and discharge will probably give some indication of the existence of malignant œdema. The tissues are softened, œdematous, and discoloured, and soaked with

a foul-smelling, sanguineous fluid, which may be frothy from the development of gas. Other bacilli will probably be present.

1. Make cover-glass specimens from the discharge. Stain some with Löffler's blue, and others by Gram's method. Examine microscopically, and look for bacilli of the form described and decolourising by Gram's method.

2. Inoculate two guinea-pigs subcutaneously with the discharge or with portions of the tissues. If the animals die, look for the characteristic organism.

3. An attempt may be made to isolate the bacillus by anaërobic cultures and plate cultivations.

SYMPTOMATIC ANTHRAX.

Symptomatic anthrax, or Rauschbrand, is a disease affecting sheep and oxen, and is unknown in man. In England it is termed colloquially 'black-leg' or 'quarter-evil,' the name being derived from the dark discolouration of the muscles of the leg and quarters of the affected animals. When the muscles are cut into a sanguineous discharge exudes, and in this are found slender bacilli, some of which are swollen or club-shaped from the presence of spores.

The bacillus of symptomatic anthrax, or *clostridium Chauveæi*, is a slender rod, never forming long threads, is strictly anaërobic and motile, but losing its motility in the presence of oxygen. It stains by the ordinary anilin dyes, but not by Gram's method. It forms endogenous spores, the spore-bearing rods being enlarged or club-shaped, hence its name of clostridium.

It can be grown in deep stabs in gelatin and agar. Gelatin is rapidly liquefied. In sugar-agar it forms a thick, irregular, greyish growth, with much development of gas. The guinea-pig is susceptible if inoculated subcutaneously or into the muscles, the bacilli being found at the seat of inoculation, but not in the blood or internal organs. Artificial immunity can be induced in various ways, by bacilli

attenuated by successive cultivations or by heat, or by heating the dried muscle to 85° to 90° C. for six hours (Kitt), also by inoculating the susceptible animal at the tip of the tail. Hanna,¹ by growing the organism in a mixture of blood plasma and broth, obtained toxins which, by careful injection, conferred immunity on rabbits, the animals after injection yielding an antitoxic serum.

CLOSTRIDIUM BUTYRICUM.

An anaërobic organism occurring in milk and producing a marked butyric acid fermentation. It forms short rods, and also long ones 3 to 10 μ in length, and filaments are met with. Spore formation takes place freely in enlarged segments. It forms a whitish growth on agar, and gelatine is rapidly liquefied, a scum forming on the surface.

¹ *Journ. Path. and Bact.* iv. 1897, p. 383.

CHAPTER XVI.

THE BLASTOMYCETES.

Saccharomycetes and Torulæ—The Common Yeasts and their Examination—The Pathogenic Blastomycetes.

THE blastomycetes, or yeasts, are sharply distinguished from the bacteria by their mode of reproduction. Whereas reproduction in the bacteria is by fission or simple division, in the blastomycetes it is by gemmation or budding. If a cell of ordinary brewer's yeast be watched under conditions favourable to growth and reproduction, it will be found that a slight prominence or pimple makes its appearance at one pole of the organism; this increases in size, and ultimately a daughter-cell resembling the parent is reproduced and separates off.

In some of the yeasts there is also a method of reproduction by endospore formation, and according as this occurs, or not, the blastomycetes are divided into two groups:

- | | | |
|---------------|---|--|
| Blastomycetes | { | 1. Saccharomycetes, or true yeasts, in which spore formation occurs. |
| | | 2. Torulæ, in which no spore formation has been observed. |

Although the term torula has thus a definite signification, it is often loosely used to denote any yeast cell.

In addition to reproduction by gemmation, the blastomycetes are also distinguished from the bacteria by their larger size, and in those forms in which endospores occur by the

spores being multiple and not single in each cell. From the hyphomycetes, or moulds, the blastomycetes are distinguished by being unicellular, and by the reproduction being asexual. The blastomycetes, however, are probably much more nearly allied to the hyphomycetes than are the bacteria, for many of the moulds have a stage in which the mycelium (see next chapter) resembles an aggregation of yeast cells, and the yeasts in old cultures form films in which the cells become much elongated, like those in the mycelium of a mould. Jörgensen and others have attempted to show that some of the yeasts are stages in the development of a fungus, but it cannot be said that this has yet been satisfactorily demonstrated.

The yeasts are of great importance in inducing many chemical changes, especially alcoholic fermentation, beer and wine being almost exclusively due to their activity.

Taking brewer's yeast, *saccharomyces cerevisiae*, as a type, the yeast cell is observed to be slightly ovoid in shape, measuring 8 to 9 μ in diameter. The bioplasm is granular, contains one or more clear spaces or vacuoles, and frequently bright, refractile globules of fatty matter, and is surrounded by a cell wall of cellulose. A nucleus is present but requires special staining to show it. When the yeast cell is freely supplied with nutriment reproduction by gemmation proceeds rapidly, and a whole string of cells may form owing to the daughter-cells budding again before they have separated from the parent. When the cell is starved gemmation ceases, fat globules and vacuoles increase in number, and the cell may finally become little more than a large vacuole, the bioplasm forming a thin coating over the inside of the cell wall. Within the vacuoles are often seen minute spherical bodies of a doubtful nature in rapid movement. Under ordinary circumstances endospore formation does not occur, but by deprivation of nutriment, as by growing on a block of plaster of Paris, the cells develop spores. First the cell becomes divided into several chambers

by the development of membranes, the so-called 'partition-wall formation,' in which the spores form. In the different yeasts the number and arrangement of the spores vary; in the *S. cerevisiae* the typical number is four, arranged close together, three on one plane and one at the top, like a pyramid of billiard balls.

In *saccharomyces cerevisiae* the nucleus can be easily demonstrated by careful staining in hæmatoxylin, Hartog's double stain of nigrosin and carmine, or by staining in anilin-water solution of gentian violet. It appears to consist, in the majority of cases, of a homogeneous substance, spherical in shape, placed between the cell wall and the vacuole. On the whole it resembles more than anything else the fragmentary nuclei in the older leaf-cells of *Chara*; that is, it consists of deeply stained granules embedded in a slightly less stainable matrix. The process of budding in a yeast cell is accompanied by the division of this nucleus into two. The division is a direct one, and does not take place in the mother-cell, but in the neck joining it to the daughter-cell. When about to divide the nucleus places itself just at the opening of this neck, and proceeds to make its way through it into the daughter-cell, until about half of it has passed through, when it divides completely, and the two nuclei thus formed separate from each other towards the opposite sides of their respective cells. The process of spore formation was observed in *saccharomyces cerevisiae*. In a cell about to sporulate the nucleus is found in the centre of the cell, and appears to be homogeneous in structure. When the nucleus divides, its outline becomes irregular and the granules arrange themselves in the form of a short rod surrounded by the other portion of the nucleus, which stains differently, and appears to form a structure of the nature of a spindle. The granules separate into two groups, and each group becomes a nucleus. The two nuclei thus formed again divide, and four nuclei are produced, each of which becomes the nucleus of a spore. A

small quantity of bioplasm accumulates round each nucleus, spore membranes appear, and four spores are thus formed, standing in the remainder of the protoplasm, from which ultimately the thick spore membranes are produced.¹

The spores are of considerable importance in the identification of species of blastomycetes, the form of the cells alone and the growths on culture media not being sufficiently distinctive. In fact so little can these two characters be relied upon, that in order to isolate in pure cultivation it is necessary to grow from a single cell. This can be done by making miniature plate cultivations with wort-gelatin on large cover-glasses (ruled in squares) which have been sterilised, and after the layer of gelatin has set, mounting, gelatin downwards, on a large cell on a glass slide. The preparation is then carefully examined with a one-sixth or one-eighth objective and the spots noted where single cells are situated. This is not a difficult matter on account of the comparatively large size of the yeast cells, and their position is determined by the cross-lines ruled on the cover-glass. The preparations are kept in a warm place, carefully covered with a bell-jar, and when visible colonies have developed, those which are derived from a single cell can be inoculated into tubes or flasks of a suitable culture medium.

It is found that the various yeasts form spores in different periods of time when grown under similar conditions, and on this fact is based what is known as the analysis of yeast—a most valuable method, which we owe to Hansen. The chief ‘diseases’ of beers and yeast—*i.e.* abnormal fermentations giving rise to inferior products—are due to admixture with the brewer’s yeast of certain ‘wild yeasts,’ as they are termed, chiefly the *S. ellipsoideus* and *S. pastorianus*; and, in order to detect these ‘disease’ species, the analysis consists in determining at what time ascospores appear. The mode of procedure is as follows :—

¹ This account of the structure of and spore formation in yeast cells is taken from a paper by Wager (see *Nature*, lvi. 1897, p. 600).

The yeast is sown in a flask of sterile wort, and incubated at 25° C. for twenty-four hours. The yeast revives, and from the deposit of young cells two cultures are made on plaster of Paris blocks. These cultures are kept, one at 25° C., the other at 15° C., and are examined twice daily. In an uncontaminated brewing yeast ascospores should not be detected in less than thirty hours in the culture kept at 25° C., and seventy-two hours in that kept at 15° C. The plaster of Paris blocks are sterilised by careful flaming in the Bunsen, and then placed in sterile glass capsules with lids, and sufficient sterilised water poured in to thoroughly moisten the whole of the blocks; unless this is done no growth occurs. By this method of analysis as little 'wild yeast' as one two-hundredth of the whole can be detected.

In addition to distinct species of yeasts there are also a number of varieties employed in brewing, &c. differing but slightly in morphological and cultural characters, yet producing varied results. These varieties may be divided into two groups—the surface, high or top; and the sedimentary, low or bottom fermentation forms. In this country beer is brewed by fermenting an infusion of malt with yeast, which, during the process, *rises to the surface* and belongs to the first group; while the German beers are obtained by yeast which, during fermentation, *sinks to the bottom*, and belongs to the second group. The floating of the yeast in the high fermentation process seems to be due to the attachment of minute bubbles of carbonic acid gas to the cells, and hitherto it has not been possible to convert the one form into the other. Hansen divides the important yeasts into groups having the same general characters, and distinguishes the varieties in each by Roman numerals (I., II., &c.).

The following are the characters of some of the more important yeasts.

CEREVISLE GROUP.—These are the yeasts producing the normal fermentations resulting in beer, &c. They are round or slightly

ovoid cells, and four ascospores are produced. In old cultures long sausage-shaped or even filamentous cells may be met with.

S. cerevisiæ I. and II.—These are bottom fermentation forms in use at the Old Carlsberg Brewery; the cells of No. II. are rounder and slightly larger than those of No. I., and ascospore formation is more abundant.

There is also a top fermentation form described by Hansen (*S. cerevisiæ I. top*), which is the yeast employed in the breweries of London and Edinburgh.

The yeasts of the *cerevisiæ* group can invert cane sugar, select dextrose from lævulose, and ferment maltose, but they cannot ferment lactose, or decompose malto-dextrin.

PASTORIANUS GROUP.—These are wild yeasts. The cells are elongated or sausage-shaped, and six or eight ascospores are produced in a cell.

S. pastorianus I.—A bottom fermentation yeast producing a bitter taste in beer.

S. pastorianus II.—A feeble top fermentation form. Surface cultures on yeast-water gelatine have smooth edges, which distinguish it from the following species:—

S. pastorianus III.—A top fermentation form producing turbidity in beer. Surface cultures on yeast-water gelatin have woolly margins.

ELLIPSOIDEUS GROUP.—These are wild yeasts. The cells are usually ovoid or pear-shaped, sometimes round, rarely elongated.

Five or six ascospores are produced in a cell.

S. ellipsoideus I.—A bottom fermentation yeast occurring on ripe grapes.

S. ellipsoideus II.—A bottom fermentation yeast causing turbidity in beer.

Both the *pastorianus* and *ellipsoideus* groups resemble the *cerevisiæ* group in their chemical actions, with the exception that they are able to decompose malto-dextrin.

S. anomalus is a yeast forming small ovoid cells. It is curious in that the spores are hemispheres with a projecting rim at the base like a felt hat.

Another point in the identification of species of yeasts is

the period of formation of films. If the yeast be grown in wort with free access of air and be undisturbed, after a varying period a film composed of a sort of zooglœa mass of cells appears on the surface.

Literature on the Yeasts of Fermentation.

Jørgensen, *Micro-organisms and Fermentation*. F. W. Lyon. (Full Bibliog.).

EXAMINATION OF YEASTS.

The yeasts can be readily examined in the fresh state in hanging-drop preparations. The cells should be young or they will not be of the typical form ; a two or three days' old culture in wort or grape-sugar may be used. The yeasts grow well on the ordinary gelatin, agar, and potato, but wort-gelatin or agar is to be preferred. The elongated cells, common to all old cultures of yeasts, may be obtained from the films which form on wort cultures in wide flasks or beakers after two or three weeks.

In order to stain yeasts, a dilution of the culture should be made in a watch-glass of water, so that the cells shall be isolated ; if they form groups in the preparations they will become distorted.

If the yeasts have been grown in wort it is best, before staining, to pour off the fluid from the deposit of cells at the bottom of the flask or test-tube, add some distilled water and shake up, and allow the cells to settle again by allowing the vessel to stand for an hour, and then repeat this process of washing once more. Cover-glass specimens may be prepared in the ordinary way and stained for five minutes in Löffler's methylene blue, washed in water, dried, and mounted. Or the cover-glasses, after air-drying, may be fixed by immersion in equal parts of alcohol and ether for ten minutes, dried in the air, and stained as before. The preparations can also be stained in gentian violet or fuchsin, or by Gram's method.

Ascospores may be double stained by preparing cover-glass specimens of a sporing culture in the ordinary way, staining in carbol-fuchsin for two minutes, rinsing in water, decolourising in alcohol for half to one minute or longer if necessary, rinsing in

water, counter-staining with Löffler's blue for five minutes, washing, drying, and mounting. The spores are red, the remainder of the cells blue.

PATHOGENIC BLASTOMYCETES.

Recently organisms apparently belonging to the blastomycetes have been isolated from certain tumours, and have been regarded as having an ætiological significance in connection with malignant disease. Sanfelice has cultivated yeast-forms from fermenting fruits which, on inoculation into guinea-pigs, produced death in about a month with the formation of a tumour at the seat of inoculation and embolic growths in the spleen and liver. He has also obtained a similar yeast from an ox affected with carcinoma, which, on subcutaneous inoculation, killed guinea-pigs in about two months, and, inoculated into the peritoneum, in a month, with multiple embolic growths in the lungs, spleen, and mesenteric glands. A good deal of calcification is present in the growths, from which fact Sanfelice named this yeast *saccharomyces lithogenes*. Rabinowitch also found that some of the ordinary yeasts give rise to a tumour formation on inoculation, especially in the rabbit.

Curtis¹ obtained a yeast from an apparently myxomatous tumour in a young man. The organism was met with in two forms, free and encapsuled. The free form appeared in young agar cultures as round or ovoid cells measuring 3 to 6 μ in diameter, often showing budding. The encapsuled form was met with in the original tumour and in the tissues of inoculated animals, and occurred as a large sphere 16 to 20 μ in diameter, enclosing the yeast cell, the capsule being hyaline and 8 to 10 μ in thickness. On agar at 37° C. it formed whitish, opaque, creamy colonies in two to three days, becoming a thick creamy growth at the end of a week. On gelatin it formed white colonies or growth in four to five days

¹ *Ann. de l'Inst. Pasteur*, x. 1896, p. 449 (Refs.).

without liquefaction, and in broth a flocculent deposit, the broth remaining clear. It was aërobic, and did not grow on serum. It formed a small quantity of acetic acid and alcohol when grown in beer-wort and sugar solutions. It was not pathogenic for guinea-pigs, but inoculated into rabbits, rats, mice, and dogs, it produced tumours and caused death. The tumours to the naked eye appeared to be myxosarcomata, and in them the yeasts were found.

Busse also obtained a pathogenic yeast from a young man who suffered from a tumour of the tibia, and ultimately died with diffused growths in the bones and organs. The yeast-like cells were observed in the affected parts, and were isolated by cultivation, and the cultures, inoculated into mice and rabbits, produced death with growths in the organs. As in Curtis's case, the cells appeared encapsuled in the tissues.

Gilchrist describes a case of blastomycetic dermatitis. Small miliary abscesses were present in the rete and corium, in the pus of which the parasitic cells were observed. These were usually in pairs of unequal size, the largest measuring about $16\ \mu$, surrounded by a well-defined capsule, and containing a granular protoplasm in which a vacuole was present. Clinically, the case had been regarded as one of scrofuloderma, but no tubercle bacilli could be found.

Literature on the Pathogenic Blastomycetes.

- Curtis, *Ann. de l'Inst. Pasteur*, x. 1896, p. 449 (Refs.).
Busse, *Die Hefen als Krankheitserreger*, Hirschwald 1897 (Full Bibliog.).
Gilchrist, *Johns Hopkins Hosp. Bull.* i. 1896, p. 269.
Gilchrist, *Journ. of Exp. Med.* ii. 1898, p. 56.
Roncali, *Journ. Path. and Bact.* v. 1898, p. 1 (Bibliog.)

CLINICAL EXAMINATION (PATHOGENIC YEASTS).

The cells can be well seen in the fresh state in the teased-up tissues in water or glycerin.

Curtis recommends staining in carbol-thionine blue, and for sections, picro-carmin.

Busse's method for sections is as follows :—

1. Hæmatoxylin solution for fifteen minutes.
2. Wash in distilled water.
3. Counter-stain in weak carbol-fuchsin (1:20) for thirty minutes to twenty-four hours.
4. Decolourise in 95 per cent. alcohol for fifteen seconds to one minute.
5. Absolute alcohol, xylol, mount in Canada balsam.

Gilchrist recommends treating the sections with 10 per cent. caustic potash solution and examining in 50 per cent. glycerin without staining.

CHAPTER XVII.

THE HYPHOMYCETES—RINGWORM AND THRUSH.

THE hyphomycetes are an important group of the true fungi, and include those forms which are commonly known as moulds. They are multicellular individuals, composed of filaments which may be simple or branched, jointed or unjointed. These filaments are termed hyphæ, and are formed by the end-to-end union of elongated cells. When the hyphæ project upwards into the air they are known as aërial hyphæ, and when downwards into the fluid or medium on which the organism is growing, as submerged hyphæ, and the compact tufts or masses resulting from numbers of interlacing hyphæ are termed mycelia. A mycelium may form a hard wooden mass or pseudo-parenchyma, which is known as a sclerotium, such as is met with in ergot. In addition to being multicellular, the higher development of the hyphomycetes is seen in the specialisation of certain parts for the function of reproduction, reproductive organs being produced. Although all the species multiply asexually, in most, if not in all, a sexual method occurs also. *Mucor mucedo*, *penicillium glaucum*, and *aspergillus niger* may be taken as types and more fully described.

MUCOR MUCEDO.

Mucor mucedo, the common white mould which appears like tufts of cotton-wool on various substances, may be obtained by exposing some moistened bread or horse dung to the air for a short time and then keeping it moist under a bell-

jar. It consists of a mycelium composed of hyphæ, and its fluffy appearance is caused by aerial hyphæ. The aerial hyphæ are at first of even diameter throughout, but later on their free ends become swollen and ultimately form spherical bodies, which become filled with spores and are known as sporangia. In the early stage the whole organism forms but a single cell, the bioplasm of which is granular, and contains vacuoles and numerous small nuclei. As it grows, and the sporangia form, these become separated by a septum from the hyphæ, and when it becomes older still the mycelial hyphæ may be divided into elongated cells. The development of a sporangium takes place as follows:—The distal end of an aerial hypha swells, and immediately below the swollen part a division occurs in the bioplasm and a cellulose septum is formed, so that the swollen part is separated off from the rest of the hypha, forming the rudimentary sporangium. The sporangium continues to grow and its bioplasm undergoes multiple fission into numerous ovoid masses, the spores, each of which becomes surrounded with a cellulose capsule. The septum separating the sporangium from the hypha projects upwards into the interior of the sporangium as a club-shaped knob known as the columella. When the sporangium is ripe the slightest touch causes its wall to rupture, so liberating the spore. When placed under favourable conditions the spore germinates, it becomes granular, and bulged out in one or more places, and these buds increase in length and ultimately form hyphæ.

Occasionally a process of conjugations occurs. Two adjacent hyphæ send out lateral branches which come in contact with one another, and a septum forms in each, separating a small portion of bioplasm from the rest of the hypha. The opposed walls of the two cells become absorbed and the contents mingle. The mass of bioplasm so formed becomes surrounded with a thick cell-wall, giving rise to an inactive spore-like body known as a zygospor. The zygospor under

favourable conditions sprouts like an ordinary spore, forming a mycelium which develops sporangia bearing aërial hyphæ.

PENICILLIUM GLAUCUM.

This forms the bluish-green mouldy patches familiar to everyone. It is by far the commonest species, and may be obtained from moist bread or jam or by exposing a gelatin plate to the air for a short time. If the mouldy patch be rubbed a fine greenish dust comes away. This dust consists of myriads of spores; if a little of it be transferred with a moistened needle to a gelatin plate or, better still, to a hanging-drop preparation, the growth of the organism can be studied. After two or three days little white specks will be observed, which microscopically are found to consist of tufts of delicate interlacing filaments, the hyphæ, which, becoming much interwoven, ultimately form a tough mycelium. The patches of growth are circular, and the hyphæ will be found to radiate from the centre. As the patch increases in size it changes in colour, becoming bluish-green, though the margin for some time still remains white. From the upper surface of the mycelium delicate aërial hyphæ grow upwards, and from the under surface short submerged ones project downwards.

The hyphæ are composed of elongated cells arranged end to end, the cell-walls of which consist of cellulose enclosing a more or less vacuolated bioplasm containing several nuclei.

The aërial hyphæ are unbranched filaments, but as development proceeds the distal ends branch dichotomously, the branches remaining short and nearly parallel to each other so that a sort of brush is produced. The ultimate branches are known as sterigmata. The ends of the sterigmata become constricted so that a little globular mass or spore is formed; this process is repeated until a chain of spores results, *the proximal one being the youngest*. A spore when placed under favourable

conditions germinates, a little bud appearing, elongating, and forming a hypha just as in mucor.

Brefeld, by sowing spores on moist bread, inverting the bread, and examining at intervals, observed a sexual method of reproduction in penicillium. Two sets of spiral cells develop on a thick hypha, they intertwine, their contents probably mingle, and from the union or carpogonium a tube-like hypha develops, which becomes surrounded and enclosed by branching hyphæ from the mother cell. By further development and thickening of the cell-walls a sclerotium form arises; it is a hard solid body, yellowish in colour and resembling a grain of sand, the carpogonium being at the centre. If placed under favourable conditions the sclerotia germinate after some time. Two forms of hyphæ are produced, one thick, the other thin; the latter become much twisted. The thick hyphæ become branched, and ultimately a number of pear-shaped bodies are produced. The contents of these bodies then become broken up and form spores; the bodies are known as asci and the spores as ascospores. From the ascospores the ordinary mycelium of penicillium again develops.¹

ASPERGILLUS NIGER.

Aspergillus (several varieties) is occasionally met with; it can be recognised by the rounded sporangia with radial markings; these are supported on aerial hyphæ given off from the mycelium, and a process of reproduction occurs very like the sexual one in penicillium. *Aspergillus niger* grows well on the ordinary laboratory media, on potato producing, after a time, a sooty growth.

With the exception of the ringworm and allied fungi, which produce parasitic skin affections, the hyphomycetes are not of very great pathological importance. In the ear

¹ See Brefeld, *Quart. Journ. Microscop. Soc.* xv. p. 342.

and nose, mucors and aspergilli may be met with, but in these situations they are epiphytes rather than parasites, and the same species occur in bronchiectases and pulmonary vomicæ. Occasionally, however, a pneumo-mycosis¹ has been met with, the mycelium of the fungus ramifying in the lung tissue and setting up irritative and other changes. The species met with in this condition seems generally to have been the *aspergillus fumigatus*.

Allusion has already been made to the possible dependence of the black variety of madura foot on a fungus.

CULTIVATION AND EXAMINATION.

The hyphomycetes can be cultivated on the ordinary laboratory media, but wort-agar, or wort-gelatin, potato, bread, or maltose agar are to be preferred.

They can be examined by removing a portion of the growth, teasing up gently with needles in a little 50 per cent. alcohol containing a trace of ammonia, removing the surplus fluid with blotting paper, and mounting in Farrant's solution or in glycerin jelly. If desired, they may be stained by the irrigation method with fuchsin.

In the tissues they may be stained with hæmatoxylin or methylene blue, or by Wigert's method.

RINGWORM.²

The ringworm fungi must probably be included in the group of the hyphomycetes. Human ringworm, formerly regarded as a simple disease, is now proved to comprise at least two affections through the researches of Sabouraud.

¹ Arkle and Hinds, *Trans. Path. Soc. Lond.* vol. 47, 1896, p. 8 (Bibliog.). Boyce, *Journ. Path. and Bact.*, Oct. 1892 (Bibliog.).

² On Ringworm, see Colcott, Fox and Blaxall, *Brit. Journ. of Dermat.* 1896 (Bibliog.), and *Trans. Path. Soc.* vol. 48, 1897, p. 301; also Malcolm Morris, *Trans. Internat. Congress of Dermatology*, 1896. Also *Brit. Med. Journ.* 1897, II.

These two forms are separated from each other both clinically and by differences in the parasitic organisms.

The first variety is an affection of early childhood, forming 80 to 90 per cent. of the ringworms met with in London; it never attacks the scalp of adults, never affects the beard or nails, is very intractable, and frequently epidemic. The parasite is characterised by small spores measuring 3 to 4 μ in diameter, and round or ovoid in shape. Affected hairs are generally broken off, forming relatively long

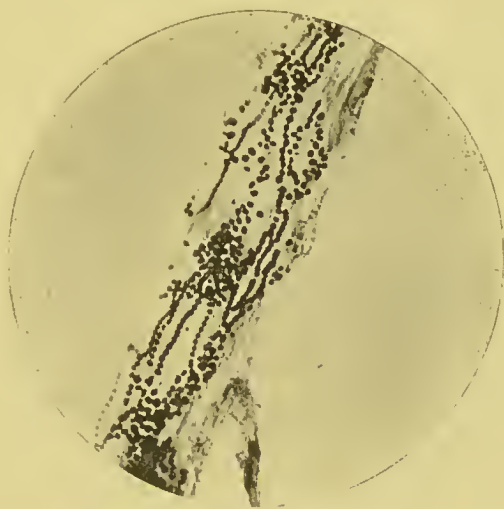


FIG. 69.—RINGWORM IN A HAIR. $\times 350$.

stumps, greyish in colour, and possessing a whitish sheath. When suitably prepared in potash, this sheath is seen to be composed of the spores agglomerated together without apparent order, and the hairs themselves are filled with delicate parallel mycelial threads. The parasite is named the *microsporion Audouini*.

The second variety comprises the ringworms with large spores, and is divided into two groups by Sabouraud. The first of these groups is exclusively of human origin, and has a marked tendency to affect the interior of the

hair only, and hence the parasite has been termed the *trichophyton megalosporon endothrix*. The other group is of animal origin, and the spores are met with chiefly on the outside of the hair, and the fungus is hence termed the *trichophyton megalosporon ectothrix*.

The *endothrix* form occurs later in childhood, is not so persistent as the *microsporon*, and does not attack the nails or beard. Microscopically, the fungus is seen to consist of beaded threads, which are the rounded or ovoid spores joined end to end. The *ectothrix* form rarely attacks the scalp, but is responsible for all the tinea sycosis and ringworm of the nails and half the cases of tinea circinata. Suppuration is common in this form. Microscopically, appearances differ; generally the spores are arranged in chains, but the sporulation is less regular than in the *endothrix*. The spores in the *endothrix* and *ectothrix* varieties measure 4 to 12 μ in diameter.

The ringworm fungi can be readily cultivated on all the ordinary media—beer-wort agar and gelatin being especially favourable. They form whitish fluffy growths with rapid liquefaction of gelatin. In order to obtain cultivations the diseased hairs or stumps are removed by forceps and placed on a sterile glass slide. The aërial portion of the hair is then cut away by means of a sterile scalpel, and the diseased portion divided into small fragments. These can be picked up with a moistened platinum needle and transferred to the culture media, preferably beer-wort agar. In some cases a pure culture is thus obtained, but in others further treatment is necessary. When the *trichophyton* or *microsporon* has thrown up its aërial hyphæ the plug of wool is removed from the tube and the mouth well flamed; the tube is then held inverted over a Petri dish containing solidified maltose agar. A sharp tap or two is given to the tube, sufficient to cause the spores to drop, and the dish re-covered. A growth of the organism from single isolated spores thus ensues, and pure cultures can be obtained (Blaxall).

The various forms of the ringworm fungi can be differentiated by cultures, but it is necessary when comparing them to employ media of identical composition, because slight differences in the latter are liable to induce marked changes in the characters of the cultures. A favourite medium, used by Sabouraud and Blaxall, is maltose agar :—

Peptone	0·5 gram
Maltose	3·8 grams
Agar-agar	1·3 grams
Water	100 c.c.



FIG. 70.—CULTURE OF THE RINGWORM ORGANISM.
ENDOTHRIX FORM.

Blaxall found that different maltoses materially influenced the characters of the cultures.

Characters of the Cultures.—Cultures are incubated at 30° C. The colonies of the *microsporon* do not show any growth until about the seventh day; little white, downy tufts then appear. The fully developed growth on maltose agar forms a large white, downy patch with a small central boss; on potato white, downy patches with brown discolouration.

The *endothrix* variety commences to grow in six or seven days, and on maltose agar in about a month forms a rounded patch with a central crateriform depression, the whole being dusted with fine white powder (fig. 70); on potato, powdery stars tinged with yellow and usually without discolouration.

The cultures of the *ectothrix* form are variable. They commence on the third or fourth day; some form whitish, smooth, or wrinkled growths; others, from the dog, form dry, brown, wrinkled, powdery growths; others, of bird origin, form purplish growths.

Microscopically, all the fungi show masses of mycelial threads with spores. They stain with the ordinary anilin dyes and also by Gram's method, and can be mounted in glycerin jelly in the manner described at page 320.

Macfadyen¹ found that the ringworm organism produced an active peptonising enzyme, and seemed to increase the solubility of keratin when grown on it; no inverting enzyme could be isolated.

CLINICAL EXAMINATION.

The hairs should be first treated with ether and then with caustic potash solution of about 7 per cent. strength. In this reagent they may remain for from a few hours to a few days; they are then floated on to a slide and carefully covered with a cover-glass. Permanent preparations may be mounted in Farrant's solution or in glycerin jelly.

Malcolm Morris gives the following method for staining the organism in the hairs:—

1. The hair is first steeped for one to two minutes in a mixture of a 5 per cent. alcoholic solution of gentian violet (one part), with anilin water (three parts).
2. Dry with blotting paper.
3. Treat with Gram's iodine solution for one to two minutes.
4. Dry again.
5. Treat with iodine in anilin oil.

¹ *Journ. of Path. and Bact.* 1895, April, p. 176.

6. Clear with anilin oil.
7. Rinse in xylol.
8. Mount in Canada-balsam.

THRUSH.¹

Thrush is due to an organism (*oidium albicans*) which is usually classed among the hyphomycetes. It forms the whitish patches so frequently seen on the mucous membrane of the mouth and pharynx in children, and in those suffering from wasting diseases. Occasionally it has produced a general infection. If one of these patches be removed and teased up it will be found to consist of masses of tangled mycelial threads with yeast-like budding. The organism can be readily cultivated on all the ordinary laboratory media, and will also grow on slightly acid media such as wort-gelatin. It produces whitish, membranous, adherent growths in which it appears morphologically under two forms, as masses of tangled filaments or hyphæ and as yeast-like cells. On acid media the latter exclusively occur, on alkaline the former predominate. It liquefies gelatin, stains by Gram's method, produces an alkaline reaction by the formation of ammonium carbonate, and does not ferment lactose. Inoculated on to a damaged mucous membrane the 'thrush' patches appear, subcutaneously it produces an abscess, and injected into the peritoneum a general infection, followed by death and accompanied by a sero-purulent peritonitis.

CLINICAL EXAMINATION.

The patches may be teased up and examined in the manner described for the hyphomycetes (p. 320). Cover-glass preparations may be stained with carbol-fuchsin or by Gram's method.

¹ See Teissier, *Arch Med. Exper.* ix. 1897, p. 253.

CHAPTER XVIII.

THE PROTOZOA.

The General Structure of the Protozoa—The Chief Parasitic Protozoa—
The Coccidia—Malaria.

THE protozoa are an important group of unicellular organisms, regarded as animal in nature, and sharply and definitely distinguished from the rest of the animal kingdom, to which the names of metazoa and enterozoa are applied. The latter consist of many cells arranged in two layers—endoderm and ectoderm—around a central cavity, the enteron.

It is true that some protozoa consist of aggregates of cells, and should therefore be entitled to be called multicellular; yet an examination of the details of structure of these cell-aggregates and of their life-history establishes the fact that the cohesion of the cells in these instances is not an essential feature of the life of such multicellular protozoa, but a secondary and non-essential arrangement. Like the budded 'persons' forming, when coherent to each other, undifferentiated 'colonies' among the polyps and corals, the coherent cells of a compound protozoon can be separated from one another and live independently; their cohesion has no economic significance. Each cell is precisely the counterpart of its neighbour; there is no common life, no distribution of function among special groups of the associated cells, and no corresponding differentiation of structure. As a contrast to this, we find in the simplest enterozoa that the cells are functionally and structurally distinguishable into two groups—those which

line the enteron or digestive cavity, and those which form the outer body wall. The cells of these two layers are not interchangeable, but are fundamentally different in properties and structure. (Ray Lankester).

By far the larger number of protozoa are absolutely single isolated cells.

A protozoon individual is a single corpuscle of bioplasm of variable size from $\frac{1}{1000}$ in. to 1 in. diameter. A nucleus is probably generally present. A large number form no cortical substance; their protoplasm is practically of the same character throughout; while an almost equal number form a distinct cortical layer of denser bioplasm, which is permanent and gives the body a definite shape.

According to Bütschli, the protozoa may be divided into the following groups:—

1. Sarkodina, forms which are amœboid.
2. Mastigophora (flagellata), non-amœboid, and moving by means of one or more long processes or flagella.
3. Infusoria (ciliata), non-amœboid, and moving by means of a few or many short processes or cilia.
4. Sporozoa, exclusively parasitic, rarely amœboid, and possessing neither flagella nor cilia. Reproduction by simple division, which occurs in the other three groups, has not been observed in the sporozoa, reproduction taking place by a process of spore formation.

Literature on the Protozoa.

- Ray Lankester, *Encyclopædia Britannica*. 9th ed. Art. 'Protozoa' (Bibliog.).
- Saville Kent, *A Manual of the Infusoria*, 1880 (Bibliog.).
- " " *Pop. Science Rev.* N.S. Vol. II., p. 113.
- " " *Pop. Science Rev.* N.S. Vol. IV., p. 293.
- C. Addison, 'On certain Protozoa occurring in the Intestine,' *Yorkshire Quart. Med. Jour.* iv. 1896 (July), p. 347 (Bibliog.).
- R. Leuckart, *The Parasites of Man* (Trans.) 1886.

GROUP I.—SARKODINA.

THE AMŒBA COLI.

The *amœba coli* is by far the most important member of this group, and was first observed by Lewis and Cunningham in the fæces in dysentery, and fully described by Lösch. It has now been met with by various observers in all parts of the world; in the fæces in cases of dysentery, and in the pus of the so-called tropical abscesses of the liver. It occurs as a large mass of bioplasm, measuring 30 to 40 μ in diameter, possessed of slow amœboid movement, and having a clearer outer zone or ectosarc and a granular endosarc. In the latter highly refractile granules occur, and it often contains blood corpuscles and a vacuole. A nucleus can also be demonstrated, but does not stain with the ordinary basic anilin dyes. Cultivation experiments have failed, but some amount of success seems to have followed inoculation into dogs. Its large size and amœboid movement, especially when observed on a warm stage, are sufficient to distinguish the organism. The presence of the *amœba coli* in the pus, and especially in the wall, of tropical abscesses, is of considerable diagnostic significance, and the parasite is considered to be of ætiological importance in cases of true tropical dysentery. The amœbæ are sometimes present in large numbers in the stools, and in the true tropical abscess the ordinary pyogenic organisms are absent.

Amœbæ are sometimes found in the intestine in health, but they are much smaller forms than the *amœba coli*.

There are, however, dysenteric conditions which are certainly not due to the *amœba coli*, but are of bacterial origin.

Ciechanowski and Nowak have obtained a bacillus belonging to the colon group, which they term the *bacterium coli dysentericum*, and a streptococcus, which they consider

to be of ætiological importance in certain cases of sporadic dysentery.

CLINICAL DIAGNOSIS.

1. A drop of the dysenteric discharge (the mucous portions should be chosen from the stools), pus, or, better, a scraping from the wall of the abscess, diluted, if necessary, with a little warm (37° C.) normal saline solution, is placed on a slide, covered with a cover-glass, and examined microscopically with a $\frac{1}{5}$ or $\frac{1}{8}$ inch objective. The amœba will be readily recognised, and may be examined more critically with a $\frac{1}{12}$ inch oil immersion. To be certain that the bodies are amœbæ, the amœboid movements must be observed by keeping the preparation on a warm stage.

2. Preparations may be stained by irrigation with methylene blue and Beale's carmine; the latter stains the nucleus, the former does not. The preparation may be rendered permanent by washing away the excess of stain, and running in some 50 per cent. glycerin by irrigation.

Mallory¹ suggests the following methods for staining the amœbæ, which he states give a differential stain, rendering the recognition of the parasite certain :—

(1) For sections (liver, intestine, hardened dysenteric discharges, &c.) :—

(a) Fix tissues in alcohol.

(b) Stain sections (paraffin) in a saturated aqueous solution of thionin for five to twenty minutes.

(c) Wash in water.

(d) Differentiate in a 2 per cent. aqueous solution of oxalic acid for half to one minute.

(e) Wash in water.

(f) Dehydrate in 95 per cent. alcohol.

(g) Clear in oil of bergamot.

(h) Wash with xylol and mount in xylol balsam.

The nuclei of the amœbæ are stained brownish red, other nuclei blue. Coverslip preparations proved a failure.

(2) Unna's differential stain :—

(a) Harden in alcohol.

¹ *Journ. of Exper. Med.* ii. 1897, p. 529.

(b) Stain sections in Unna's polychrome methylene blue solution (Grübler's) quarter hour to all night.

(c) Decolourise and differentiate in a small dish of water, to which are added a few drops of Grübler's glycerin-ether mixture.

(d) Wash well in water.

(e) Alcohol, oil of bergamot, and mount as in 1.

This may give better results with the discharges.

Literature on Dysentery.

Councilman and Lafleur, *Johns Hopkins Hosp. Reps.* ii. 1891, p. 395 (Full Bibliog.).

Ciechanowski and Nowak, *Centr. f. Bakt.* xxiii. 1898, pp. 445, 493 (Bibliog.).

Allusion may here be made to the mycetozoa. These are masses of bioplasm resembling huge amœbæ, and are found on decaying vegetable matter. They are now usually regarded as vegetable in nature, and some important plant diseases, such as the 'finger and toe' of cabbage roots, are due to their activity.

GROUP II.—MASTIGOPHORA (FLAGELLATA).

Several interesting animal parasites belong to this group.

HERPOMONAS LEWISII.

This parasite was discovered by Lewis in the blood of Indian rats and by Crookshank¹ in the blood of the common sewer rat. It is an elongated spindle in shape, 20 to 30 μ in length and about 1 μ in breadth, terminating in a spine-like process at one end, and having a delicate flagellum at the other of about the same length as the body. The bioplasm is granular, and contains one or more highly refractile spherules, and a delicate undulating membrane is

¹ *Journ. Roy. Microscop. Soc.* 1886.

attached to one aspect like a dorsal fin. It moves by means of the flagellum in the direction of the flagellated end. Crookshank found it in 25 per cent. of the rats he examined.

In a disease known as 'Surra,' affecting horses, mules, and camels in India and Burma, and characterised by fever, jaundice, and wasting, a parasite morphologically identical with the *herpomonas Lewisii* has been found by Evans. The disease can be transmitted by inoculation to other animals of the same species, and also to the dog and monkey.

Very similar parasites have been observed also in the tsetse-fly disease, and in many fish, such as the carp.

TRICHOMONAS VAGINALIS.

This parasite is found in the acid vaginal mucus in 50 per cent. of those examined. It must not be mistaken for a spermatozoon. It is a pear-shaped body, measuring 12 to 30 μ in length, and from the blunt end four flagella are given off.

A much smaller species, *T. intestinalis*, measuring 4 to 15 μ , has been met with in the intestinal canal of man in conditions associated with diarrhoea.

GROUP III.—INFUSORIA (CILIATA).

BALANTIDIUM (PARAMÆCIUM) COLI.

This is an intestinal parasite of swine, occasionally met with in man in conditions associated with chronic diarrhoea.

It is somewhat ovoid in shape, the ends being bluntly pointed, is covered with cilia, and measures 65 to 85 μ in length.

This organism has assumed some importance since Klein in a report on the London water supply stated that he had found it in all the waters examined, and 'that it is of considerable size, and is clearly derived from sewage-polluted

water, being almost a normal inhabitant of faecal matters.'¹ It is important, therefore, to distinguish it from the ordinary water paramœcia which might well be met with in the water mains under normal conditions. Leuckart, however, states that the *B. coli* has not been observed in Germany, France, or England.

According to Saville Kent, the *balantidium coli* is to be distinguished from the ordinary forms of water paramœcia by the following characters. The *B. coli* is somewhat spindle-shaped or ovoid, and bluntly pointed at each end, one and a half to twice as long as broad, measuring $\frac{1}{360}$ inch to $\frac{1}{168}$ inch in length; the paramœcium is more cylindrical, four times as long as broad, measuring $\frac{1}{120}$ inch to $\frac{1}{96}$ inch in length. The oral aperture in *B. coli* is near one extremity; in paramœcium it is situated at about the middle of the ventral surface. In *B. coli* the cilia round the oral aperture are as long again as those over the body generally; in paramœcium the whole of the cilia are of the same length.

EXAMINATION OF FLAGELLATED AND CILIATED FORMS.

1. These may be examined fresh in the fluid in which they are present, by mounting on a slide, and covering with a cover-glass, one edge of which rests on a bristle to avoid pressure.

2. Permanent mounts may be made by irrigating with Beale's carmine, washing the stain away with weak glycerin, and mounting in 50 per cent. glycerin.

3. Cover-glass preparations may be made in the ordinary way, and the films stained with gentian violet or weak carbol-fuchsin. (The organisms are apt to be distorted.)

4. The following method, devised by Rousselet (*Journ. Quekett Microscop. Club*, 2nd Series, vi. No. 36, p. 5, March 1895), for preserving rotatoria might be tried. In those forms which are non-contractile, kill by adding a drop of $\frac{1}{4}$ per cent. osmic acid, wash

¹ 'Analytical Investigation of the London Water Supply': *Report to the London County Council*, 1896, p. 14.

immediately in water, and preserve in $2\frac{1}{2}$ per cent. formalin. Contractile forms may be first narcotised by adding a drop or two of 2 per cent. cocaine solution, then killed with the osmic and preserved as before.

GROUP IV.—SPOROZOA.

This is by far the most important group of the parasitic protozoa, and includes several parasites producing important diseases.

COCCIDIAL DISEASE OF RABBITS.

This is a disease caused by a sporozoon, the *coccidium oviforme*, and often met with in warrens and hutches; in some of the former as many as 90 per cent. of the animals may be affected. The young animals suffer most, and become infected when they cease to suckle and commence to eat green food, the adult animal as a rule resisting the disease. The affected animals waste, suffer from enteritis, and a large proportion die in from 1 to 3 weeks, the condition being known as 'wet-snout' among the keepers. The parasites occur in the intestine, bile ducts, and liver in large numbers. Each parasite is ovoid in shape, measuring $36\ \mu$ in length and $22\ \mu$ in breadth, is enclosed in a firm translucent cyst, which encircles a very granular bioplasm. Sometimes this bioplasm becomes condensed so as to form a spherical mass lying free within the cyst. In the intestine and bile ducts the parasites are attached to the epithelial cells, and in the liver, if the animal lives beyond the acute stage, set up some remarkable changes. The affected liver is studded with greyish-white nodules varying in size from a pin's head to a pea. On making sections and examining them microscopically it is found that these nodules consist of dilated bile ducts filled with a much hypertrophied and convoluted mucous membrane, which forms branched projections covered with

cubical epithelium, amongst which the parasites occur in great numbers. A curious fact is that subcutaneous or intravenous inoculation, or inoculation into the liver of a healthy rabbit with the coccidia from another rabbit, fails to induce the disease.

The coccidia have a complicated life-history, and infection only seems possible in one of the stages. In order to study the life-cycle the parasite must be placed under suitable conditions, and an infusion of rabbits' faeces, kept at the ordinary temperature, is perhaps as good a cultivating medium as any, the changes being watched by means of interlamellar films. When the coccidia are observed under these conditions, the first change is the condensation of the bioplasm so as to form a sphere lying free within the cyst, a stage sometimes observed in the animal. The sphere then divides into four smaller spherules. Each spherule becomes elongated, and again divides into two somewhat crescent-shaped bodies, around each pair of which a new, somewhat spindle-shaped capsule forms. In this condition the parasite is very resistant, and may remain alive for six months, undergoing no further change unless introduced into another animal. If a young rabbit swallows with its food these crescentic spores, the enclosing capsule is dissolved, and each crescent becomes a rounded amoeboid mass, and this again divides up into many crescentic spores. These spores are apparently motile, and enter the epithelial cells of the intestine, gall bladder, and bile ducts, where a process of growth and differentiation occurs, and the fully developed parasite is ultimately reproduced.

Some chemical reactions of the coccidium were investigated by the writer,¹ and are of interest in connection with the chemistry of carcinoma. Frequently the coccidia contain one or more bright refractile bodies, which stain black with osmic acid, and are therefore probably fatty in nature,

¹ Paper read at the Brit. Med. Assoc. Meeting, London, 1895.

and here and there an organism will stain a more or less diffuse black with this reagent. With an iodine solution, such as Gram's, the coccidia from the liver stain a deep mahogany-brown, a reaction previously noticed by Malassez, Delépine, and others. This colouration is very like that given by glycogen, and the writer found that the colour of the stained coccidia disappeared on warming, and returned again on cooling, in which respect it resembles glycogen, and the reaction is probably due to that substance. The capsule of protozoa is generally supposed to consist of chitin. The writer found, however, that the capsule of the coccidium is not dissolved by concentrated sulphuric or hydrochloric acids, even after acting for days, whereas chitin prepared from shrimps is quickly dissolved by these reagents. It is unattacked by boiling caustic potash, and does not give a cellulose reaction with Schultze's solution. No abnormal distribution of phosphorus or iron can be detected by the methods of Lilienfeld and Monti and Macallum respectively. The livers affected with coccidial disease from several rabbits were examined for the presence of a proteose. They were minced, placed in absolute alcohol for some weeks, dried, and then extracted. The weight of the dried material was 47.3 grams and the dried extract from this weighed .68 gram, and in its reactions corresponded with deuterio-albumose. A dose of 0.1 gram injected subcutaneously into a small guinea-pig produced no appreciable effect.

Coccidial disease, or, as it is sometimes termed, psorospermiosis, is occasionally met with in animals, as the sheep, and a wasting disease of young pheasants due to coccidia has been described by MacFadyean.

In man, coccidial disease has been described (but rarely) in the liver, gall bladder, ureter, &c.

Rixford and Gilchrist describe two cases of protozoon infection of the skin and organs, accompanied by great destruction of tissue and ending in death. The organisms

were spherical, 7 to 27 μ in diameter, surrounded by a thick capsule, enclosing granular bioplasm.

Cancer, molluscum contagiosum, Paget's disease of the nipple, &c. have been ascribed to sporozoa. These conditions will be considered in Chapter XIX.

Literature on Coccidia.

- L. Malassez, *Archiv. de Méd. exper. et d'Anat. Path.* iii. 1891, p. 1.
 S. Delépine, *Trans. Path. Soc. Lond.* 1890, xli. p. 346 (Bibliog.).
 Galloway, Morton Lect. on Cancer, *Brit. Med. Journ.* 1893, i. p. 217 (Bibliog.).
 J. MacFadyean, *Journ. Comp. Path. and Therapeut.* 1895.
 Rixford and Gilchrist, *Johns Hopkins Hosp. Reps.* i. 1896, p. 209.

CLINICAL EXAMINATION.

1. The coccidial forms are readily examined in the fresh state. The only bodies they are likely to be mistaken for are certain ova.

2. Sections containing rabbit coccidia, &c. (paraffin are best) may be stained much in the same way as tubercular tissues—viz., warm carbol-fuchsin ten minutes, decolourise *cautiously* in 25 per cent. acid, and counter-stain in methylene blue. Sections may also be stained in the Ehrlich-Biondi stain for one to two hours.

PEBRINE.

Pébrine is a disease of the silkworm, of considerable importance commercially, for the silk industry in France was once threatened with extinction owing to its ravages. It is observed in the worms; they do not grow normally, and cease to eat, die, or form abnormal pupæ. Within the body of the affected worms a large number of roundish, highly refractile corpuscles are formed. Pasteur ascertained that the disease was propagated by healthy worms eating with their food the excreta of infected ones. The moths were thus infected, and laid infected eggs. By allowing each moth to lay its egg separately, and subsequent microscopical examination of the body of the moth, he was able to separate the healthy from the diseased, and the eggs of the

former were kept, while those of the latter were destroyed. The parasite is regarded as a protozoon belonging to the sporozoa. According to Pfeiffer,¹ when the worms eat the excreta containing the corpuscles mentioned above, these lose their capsule and form large amœboid masses which penetrate the muscles and blood corpuscles. The amœboid masses then become encapsuled, and are yellow and granular. Later on the bright roundish corpuscles form within them. The parasite has been termed the *nosema bombycis*.

Another disease of silkworms is known as flacherie, but is due to a bacterium, *micrococcus bombycis*. It is contagious, and can be transmitted by inoculation.

MALARIA.

In 1879 Klebs and Tommasi Crudeli obtained a bacillus, termed by them the *bacillus malarie*, from the air, water, and soil of the Roman Campagna. The bacillus was motile, formed spores, and in fluid culture media and in the bodies of animals grew into long filaments; injected into rabbits it produced a febrile condition which was described by these observers as analogous to that of malaria. Since then a number of observations in all parts of the world have thrown discredit on the theory that the *bacillus malarie* is the cause of malaria, which disease is now believed to be due to a protozoan parasite.

The credit of the discovery of this protozoan parasite must be given to Laveran, who described it as occurring in four phases, viz.—(1) Spherical bodies, (2) flagellated bodies, (3) crescentic bodies, and (4) segmented or rosette bodies.

1. *The Spherical Bodies*.—These Laveran describes as being the commonest forms. They are hyaline and amœboid; the diameter of the smallest is about 1μ and of the largest nearly that of a red blood corpuscle. The majority of

¹ *Zeitsch. f. Hyg.* iii. 1888, p. 3.

the spherical bodies are attached to the red blood corpuscles, but some are free in the plasma; they are structureless or possess one to several dark pigment granules, sometimes central, sometimes arranged round the periphery (A, B, and C, fig. 71). These spherical bodies may divide into three or four similar ones, and exist at the expense of the blood corpuscles, which they ultimately destroy.

2. *Flagellated Bodies*.—When a specimen of blood containing free spherical bodies is carefully examined, it will be found that some of them possess one, two, three, or four

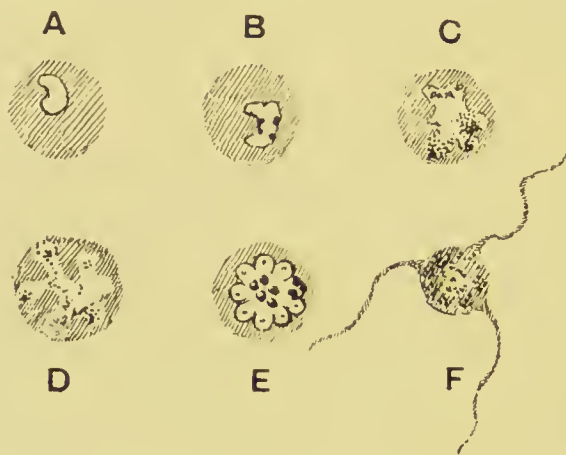


FIG. 71.— THE MALARIA PARASITE. A, B, C, Forms of the Quartan Parasite. D, E, Forms of the Tertian Parasite. F, the Flagellated Form (after Marchiafava and Brignami).

flagella, which may be 20 to 30 μ in length (F, fig. 71). The flagella move rapidly, displacing the blood corpuscles. The flagellated bodies are much more numerous in the blood of the spleen than in that of the peripheral vessels.

3. *The Crescentic Bodies*.—These vary in shape from that of a sausage to a crescent with rounded extremities; their long diameter is slightly greater than that of the red blood corpuscles, and they are structureless except for a collection of dark pigment granules about their centre (A, B, and C, fig. 72). In the truly crescentic forms the extremities of the crescent

often seem to be joined by a delicate membrane. The crescentic bodies are non-motile and are always free in the plasma, and they appear to be blood corpuscles almost entirely destroyed by the invading parasite, the membrane joining the ends of the crescent being the remains of the blood corpuscle.

4. *The Rosette Bodies* are spherical elements pigmented at the centre and regularly segmented so as to have the appearance of a rosette. They are regarded as the parasite in a sporing condition (E, fig. 71).

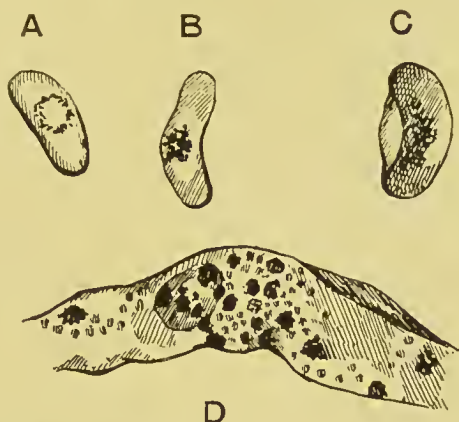


FIG. 72.—THE MALARIA PARASITE. A, B, C, Forms of the Crescentic Bodies of Laveran. D, Brain Capillary, with Pigmented Parasites from a Case of Comatose Malaria (after Marchiafava and Brignami).

The parasites cannot be cultivated, but inoculation of healthy individuals with the blood of malarial patients reproduces the disease, and the same structures or parasites are found in the blood of these infected persons. The development of the malarial parasite is described by Golgi as follows:—The free unpigmented spores for a time float in the plasma; they then become attached to the red blood corpuscles and form the amoeboid bodies. Afterwards they become enlarged and pigmented, and feed on the red blood cells, the pigment granules being composed of altered hæmoglobin.

When the parasite becomes so large as to occupy the

whole of the blood corpuscle, spore formation commences by regular segmentation so that the parasite assumes the rosette stage. The divisions of the rosette then become spherical and separate from each other, each one forming a free spore. The flagellated bodies appear in all the varieties of the parasite, and they were regarded by Laveran as representing its highest developmental stage. Grassi and Feletti considered them to be dying parasites, and Mannaberg as a stage suited to saprophytic life outside the body. The latter seems to be Manson's¹ view, and he has confirmed Marshall's and Ross's experiments on the influence of water and exposure to air in inducing flagellation. If the finger be pricked through vaseline, and the droplet of blood allowed to remain covered by the grease film for an hour or so, Ross found that flagellation did not occur, whereas if at the same time another specimen of the blood were kept in a moist chamber for the same period and then examined flagellation was found to be present. Manson's method of obtaining and staining flagellated forms will be found in the section on clinical examination.

Mannaberg regards the crescentic bodies as produced by the coalescence of the amœboid ones, but Thayer and Hewetson dissent from this. Laveran considered that the parasites met with in the various types of malarial fever belonged to the same species, but the general opinion seems now to be that there are distinct varieties, if not species.

The tertian and quartan parasites are amœboid, at the maximum size as large as, or even slightly larger than, the red blood corpuscles, and do not give rise to any crescentic bodies; in the tertian, amœboid movement is more active than in the quartan, and the spores are more numerous, fifteen to twenty in the former, six to twelve in the latter. The quotidian and malignant tertian parasites develop crescentic bodies, they never become more than two-thirds

¹ *Brit. Med. Journ.* 1897, ii, p. 68.

the size of a red blood corpuscle, and the spore formation is generally irregular instead of the regular rosettes of the tertian and quartan. Two varieties of the quotidian parasite are known, pigmented and unpigmented. In each the spores number six to eight, whereas in the malignant tertian there are usually about twelve.

In the malignant malarial fevers, which are generally of the tertian type, and end by coma, the parasites are very numerous in the capillaries of the brain (D, fig. 72) and spleen, the blood corpuscles containing large numbers of pigmented and sporing forms. Thin suggests that the abundance of parasites in the cerebral capillaries may be due to the comparatively small size of the latter. Some of the morphological differences between the varieties of the parasites in the different forms of malarial fever have been mentioned, but the essential distinction between them is the different times required for their maturation from spore, to spore again, the quartan parasite requiring seventy-two hours, the tertian parasite forty-eight hours, and the quotidian twenty-four hours.

The majority of authorities regard the malarial parasite as a protozoan belonging to the sporozoa, and it has been variously termed hæmatozoon, plasmodium, and hæmamæba.

Cultivation experiments have invariably failed, and the manner in which the parasite exists outside the body is a matter of conjecture. Amœbæ have been noticed in the soil in malarial districts, and have been suggested as being the saprophytic form of the parasite, but it is not improbable that the stage outside the human body is passed in some other animal form, perhaps an insect, such as the mosquito, a theory supported by Manson. Inoculation experiments on all animals except man have proved negative, and in the latter the inoculation must be intravenous. According to Laveran, the causal relations of the malarial parasite to the disease are based on the following considerations:—

1. The parasites have been found in the palustral patients

of all countries, they have the same characteristics, and there is a remarkable agreement between the numerous descriptions which have been given of them.

2. The parasites have never been found in persons who were not suffering from paludism.

3. The development of the parasites is intimately connected with the production of melanæmia, which is the most characteristic lesion of paludism.

4. Quinine causes the parasites to disappear from the blood at the same time as it cures the palustral fever.

5. It has been possible to transmit paludism from man to man by injecting a small quantity of blood taken from the veins of a palustral patient, and containing parasites, into the veins of a subject free from paludism.

The cure of malaria by quinine is regarded as being due to a poisonous action on the parasites analogous to that exerted on numerous protozoa, amœbæ, for example, being injuriously affected by so little as a 1-50000 solution of quinine hydrochlorate.

The infection of malaria is undoubtedly conveyed through the air, and probably also through drinking-water.

A number of blood parasites resembling those of malaria have been observed in the blood of many birds, pigeon, hooded crow, sparrow, and others, and in the frog, lizard, tortoise, and other amphibians.

CLINICAL EXAMINATION.

The blood of malarial parasites may be examined either in the unstained or stained condition.

Examination in the unstained condition.—The finger or lobe of the ear is pricked, and a droplet of blood taken up on a clean cover-glass, which is then placed upon a slide, so that the droplet of blood spreads out into a thin layer between the two glasses. The cover-glass may then be ringed with oil or vaseline to prevent evaporation. A little practice is required to judge the right

quantity of blood. The preparation should be examined with a $\frac{1}{2}$ inch oil immersion lens.

Examination in the stained condition.—To prepare stained specimens the finger or ear is pricked as before, and a droplet of blood taken up on a cover-glass ; another cover-glass is applied, and the two are separated so that each is smeared with a thin film of blood. Several are prepared in this manner, and the films are dried in the air, then fixed by passing through the flame in the ordinary way, and stained as described below. It is far better, instead of fixing in the flame, to fix in a mixture of equal parts of alcohol and ether for half an hour, and then dry between blotting paper. Manson recommends picking up a droplet of blood on an oblong slip of fine clean tissue paper. The charged surface of the paper is then applied to a clean glass slide ; in a second or so the blood will have formed a thin film between the slide and the tissue paper. The latter is then withdrawn, leaving a very thin film on the glass, and applied to a second slide, and, in like manner, to three or four in succession. The preparations may be stained in Chenzinsky's solution (p. 76) or in a half-saturated aqueous solution of methylene blue for half an hour, washing in water, and counter-staining with a 2 per cent. solution of eosin in 60 per cent. alcohol for half an hour, washing, drying, and mounting. Manson recommends treating the films with very weak acetic acid—2 or 3 drops to the ounce of water—to wash out the hæmoglobin, and, after washing, staining in the following solution for half a minute :—

Borax	5 parts
Methylene blue	0.5 part
Water	100 parts

washing, drying, and mounting in xylol balsam.

Marchoux recommends staining for a few seconds in the following carbol-thionine solution, washing, drying, and mounting :—

Saturated solution of thionine in		
50 per cent. alcohol	20 c.c.	
Aqueous solution of carbolic acid		
(2 per cent.)	100 c.c.	

This solution requires to be kept for a few days before it is ready for use.

In order to demonstrate the flagellated organisms Manson recommends the following procedure: Thirty or forty strips of thick blotting paper (3 inches by $1\frac{1}{2}$ inch), each having an oblong hole ($\frac{7}{8}$ inch by $\frac{2}{3}$ inch) cut lengthways in the centre are prepared; they are then moistened with water and laid on a sheet of window glass. A patient is selected in whose blood the crescentic form is plentiful, and a minute droplet of the blood, about the size of a pin's head, is expressed from a prick. A clean slide is then breathed on, and the droplet of blood picked up on it and spread out with a needle so as to cover an area $\frac{3}{4}$ inch by $\frac{1}{2}$ inch. The slide is immediately inverted over a blotting-paper cell and pressed down sufficiently to secure perfect apposition. The rest of the paper cells are similarly covered with blood-charged slides. In from half to three quarters of an hour the slides are removed and dried by gentle warming, and then fixed with absolute alcohol for five minutes. The alcohol is allowed to evaporate, and the films are treated with a few drops of 15 per cent. acetic acid to dissolve out the hæmoglobin. The slides are then washed in water and stained with weak carbol fuchsin (20 per cent.) for six to eight hours, washed in water, dried, and mounted.

N.B.—Negative results in the examination for the malarial parasite must be accepted with caution unless repeated. A single undoubted parasite is sufficient to establish the diagnosis. Quinine causes the disappearance of the parasite.

Literature on Malaria.

The following works and papers may be further consulted:—

- Paludism*, Laveran, New Syd. Soc. 1893 (Bibliog.).
- Parasites of Malarial Fevers*, *ibid.* 1894 (Bibliog.).
- 'The Malarial Fevers of Baltimore,' Thayer and Hewetson, *Johns Hopkins Hosp. Reps.* v. 1895, p. 5 (Bibliog.).
- 'Preparing Malarial Blood Films,' Manson, *Brit. Med. Journ.* 1896, ii. p. 122. (Also *ibid.* 1894, ii. p. 1252.)
- 'Staining Flagellated Organisms,' Manson, *Brit. Med. Journ.* 1897, ii. p. 68. (Also *ibid.* 1894, ii. p. 1306.)
- On Bird and Animal Parasites, see Opie and MacCallum, *Journ. of Exp. Med.* iii. 1898, pp. 79–136 (Full Bibliog.).

CHAPTER XIX.

DISEASES OF UNCERTAIN ETIOLOGY.

Scarlet Fever—Hydrophobia—Malignant Disease—Vaccinia and Variola.

SCARLET FEVER.

Up to the present time no micro-organism has been definitely associated with scarlet fever. Various organisms have been described in this disease—a bacillus by Eddington and a streptococcus by Fränkel and Freudenberg. A streptococcus has been claimed by Klein to be the specific infective agent, but the researches of Crookshank and others seem to disprove this.

In 1885 an epidemic of scarlet fever occurred in Marylebone, and was traced by the inspectors of the Local Government Board to infection conveyed by milk supplied from a farm at Hendon. They were unable to trace the infection to any human source, and came to the conclusion that the cows themselves were affected with scarlet fever, and so contaminated the milk. On examination, the udders and teats of the cows were found to present a vesicular eruption, and it was supposed that this was the local manifestation of bovine scarlatina. From the vesicles and crusts Klein isolated a streptococcus which, although closely resembling the *streptococcus pyogenes*, differed slightly from it; on inoculation into calves it produced death, with lesions of the kidney, &c. resembling those of the human disease. Klein also isolated the same streptococcus in five out of eleven cases of the disease in man. The conclusions which Klein and Power came to were, therefore, that scarlet fever is com-

municable to, and may exist in, cows, the milk thereby becoming infected and conveying the disease to man, and that a streptococcus is the specific infective agent.

The Hendon outbreak was reinvestigated by Axe and Crookshank.¹ Axe found that so far from there being no source of human infection, cases of scarlet fever had occurred near the dairy within a short time of the outbreak, and the eruptive disease of the cows was shown by Crookshank to be cow-pox, while the streptococcus of scarlet fever is regarded as a variety of the *streptococcus pyogenes*. Undoubtedly, scarlet fever may be milk-borne, but the infection is from a human source, and the existence of bovine scarlet fever is entirely discredited by the veterinary profession, both here and on the Continent.

Structures, stated to be protozoa, have been observed in the blood of scarlet-fever patients by some observers.

HYDROPHOBIA.

Hydrophobia attacking man is invariably contracted through the bite of an animal affected with the disease. It is most frequent in the dog, but the cat, wolf, and deer are also subject to it, and other animals can be infected by inoculation. In the lower animals the disease is termed rabies, and takes two forms, either the raging one or the paralytic. The latter is not met with in man, unless the rare forms of acute ascending paralysis (Landry's) be a manifestation of it. In the dog either may occur, and in rodents the paralytic is almost always the form it assumes. In man the incubation period is very variable; it is never less than about twenty days, and possibly may be as long as two years, or even more; the average seems to be about ten weeks. In the rabbit, after inoculation from the dog, the incubation period is about two to three weeks.

The virus resides in the central nervous system, as was

¹ On the Hendon outbreak, see *Trans. Path. Soc. Lond.* 1888 (Refs.)

shown by Pasteur, but no one has yet succeeded in isolating a specific organism, though structures have been observed variously supposed to be micrococci, protozoa, &c. Inoculation with emulsions prepared from the medulla and with the saliva conveys the disease, but the other tissues and fluids of the body are non-infective, and the filtered emulsions are also inactive.

Babes¹ states that the virus is destroyed at a temperature of 60° C., but the medulla and other infective material retain their virulence for months in glycerin.

Pasteur showed that the virus could be attenuated by desiccating the infective nerve matter, and in this way was able to prepare a vaccine which would protect animals from otherwise fatal doses of the virus. Advancing a step further, he used his vaccines to treat individuals who had been bitten by rabid animals, but in whom the symptoms had not yet developed, and so inaugurated the present system of anti-rabic inoculation as carried out at the Pasteur Institute.

To prepare the anti-rabic vaccines a rabbit is inoculated sub-durally with an emulsion prepared from the medulla of a rabid dog. When the animal dies a second rabbit is similarly inoculated from the first, and the passage through rabbits is continued until a 'fixed virus' is obtained, which kills with certainty in about seven days. This having been attained, two or three rabbits are inoculated sub-durally every day, so that there is a daily supply of animals dead of the disease. The spinal cord is removed with aseptic precautions, cut into convenient segments and suspended in bell-jars containing a layer of caustic potash at the bottom, which serves to desiccate them. The jars are dated and preserved in glass cases in a dark room, and kept at a constant temperature of about 23° C. The vaccine fluids are prepared by triturating portions of the dried cords in sterile broth, so as to form an emulsion—5 millimetres of cord in 3 c.c. of sterile broth forming a single dose. At the commencement of treatment

¹ *Brit. Med. Journ. Epit.* 1896, i. No. 56.

the cords which have been dried for fourteen days are used, at the end of treatment those which have been dried for only three days; the latter are much more virulent, and would communicate the disease but for the previous treatment. The treatment varies in duration according to the severity of the case, which is gauged by the number and situation of the bites and by the species of animal. Bites on exposed parts are regarded as much more serious than those through clothing, and on the face, where efficient treatment is difficult, than on the hands, and wolf bites than dog bites.

The doses are injected subcutaneously in the flank, and do not produce much constitutional disturbance. At first there is a feeling of lassitude and considerable muscular tenderness at the seat of inoculation, which later on passes off. Allen¹ gives the following scheme of the system and dosage employed at the Pasteur Institute at the present time:—

ORDINARY TREATMENT.		GRAVE CASES.	
Day of Treatment.	Days of Desiccation of Cord used.	Day of Treatment.	Days of Desiccation of Cord used.
1 . . .	14 and 13	Same as ordinary until 10th day.	
2 . . .	12 and 11	11 . . .	4
3 . . .	10 and 9	12 . . .	3
4 . . .	8 and 7	13 . . .	5
5 . . .	6	14 . . .	5
6 . . .	6	15 . . .	4
7 . . .	5	16 . . .	4
8 . . .	4	17 . . .	3, half dose
9 . . .	3, half dose	18 . . .	3, full dose
10 . . .	5, full dose	19 . . .	5
11 . . .	5	20 . . .	3
12 . . .	4	21 . . .	4
13 . . .	4	22 . . .	3
14 . . .	3, half dose		
15 . . .	3, full dose		
CASES OF MODERATE GRAVITY.		VERY GRAVE CASES.	
Same as ordinary until 13th day.		In addition to the previous.	
14 . . .	3	23 . . .	5
15 . . .	5	24 . . .	4
16 . . .	4	25 . . .	3, half dose
17 . . .	3, half dose	26 . . .	3, full dose
18 . . .	3, full dose		

¹ *Birmingham Med. Rev.* 1898, Jan. p. 1.

Undoubtedly the Pasteur inoculations will protect animals from rabies, the duration of immunity after vaccination in the dog being at least three years. In man the efficacy of the treatment can only be judged by statistics. The mortality after bites by supposed rabid animals is variously stated, the most favourable being about 16 per cent. (Leblanc). During 1896, 1,308 persons were treated at the Pasteur Institute with four deaths, a mortality of only 0·30 per cent. In 2,730 cases treated, in which the animal which inflicted the bites was proved to be rabid by inoculation experiments, only nineteen deaths occurred, a mortality of 0·7 per cent.

The failure of the treatment may be due to two causes : (1) delay in its commencement, and (2) a short incubation period. The principle of the treatment probably depends upon the long incubation period of the disease, owing to which it is possible to immunise the body by the inoculations before the onset. If, unfortunately, the infective material should be very virulent, and the incubation period thereby reduced to the lower limit, it may be impossible to do this before the onset of the disease, and the same is the case if the commencement of the treatment be delayed. Pasteur's system of inoculation is useless when the disease has declared itself.

By vaccinating animals by the Pasteur method by a long series of injections, and with the most virulent material, the blood serum acquires 'antitoxic' properties, and this 'anti-rabic' serum is said to be of service in the treatment of the declared disease.

DIAGNOSIS OF RABIES.

In order to ascertain whether an animal is rabid it is necessary to make use of the inoculation method. The brain of the animal is removed in as fresh a condition as possible, placed upper surface downwards in an aseptic dish, the medulla dissected away from the cerebellum and thrown forward, thus exposing the floor

of the fourth ventricle. From the middle of the floor of the fourth ventricle a small piece about the size of a pea is removed ; this is mashed up and thoroughly emulsified in a sterile watch-glass by means of a sterile glass rod with a bulbous end, a little sterile broth being used to make the emulsion, and sufficient being added to measure about 10 c.c. The hair on the head of a good-sized rabbit is cut close, the animal is anaesthetised with ether, the skin on the scalp reflected, and a trephine hole made through the skull. The centre of the trephine hole should be in the middle line, and on the line drawn between the posterior corners of the eyes ; the diameter of the trephine being about $\frac{3}{16}$ inch. A little of the emulsion is drawn up in a small syringe, having a fine needle, and two or three drops are injected beneath the dura mater. The operation is carried out with antiseptic precautions, the wound closed, and a little wool and collodion dressing applied.

If the material injected be from a rabid animal the first symptoms will be noticed in from ten to fourteen days. The inoculated animal loses control over its hind legs and throws them about peculiarly when running. This increases, and in another day or so the animal is apt to fall when running, and in another day or two the hinder extremities become paralytic, and the animal is unable to move, and dies shortly. The onset of symptoms is hardly ever delayed beyond twenty-one days.

Babes (*loc. cit.*) describes certain changes in the medulla and cord, which he states are pathognomonic. These are little hyaline globular masses about the small blood-vessels, due to their obliteration by cell-proliferation. A piece of the cord is hardened in alcohol, and stained with anilin red, and these appearances sought for.

MALIGNANT DISEASE.

The analogies between carcinoma and sarcoma and many infective diseases have led investigators to search for micro-organisms in these conditions.

Many years ago Scheuerlein isolated a bacillus in scirrhus disease of the breast, but it was afterwards shown

that the organism was one of the varieties of the *bacillus mesentericus*.

Shattock ¹ has more recently made investigations in this direction, and has been unable to isolate any bacterial form from malignant disease.

A great impetus was given to the study of parasites in malignant disease by the publication of a paper by Russell.² He observed, by certain methods of staining, small corpuscles within the epithelial cells. They were spherical in shape, 4 to 10 μ in diameter, occurring singly or in groups, were apparently homogeneous, and surrounded by a capsule. Russell



FIG. 73.—THE SO-CALLED PROTOZOON PARASITE OF CANCER. *a*, An Epithelial Cell containing Four Parasites. *b*, An Epithelial Cell containing One Large Parasite (after Ruffer and Walker).

regarded these structures as belonging to the 'sprouting fungi' (blastomycetes), and they have since been known by the name of 'fuchsin bodies' or 'Russell's corpuscles.'

More recently structures have been observed within the epithelial cells of carcinoma which have been regarded by many investigators as parasitic protozoa. These structures are round or ovoid, 2 μ to 10 μ in diameter, with a very distinct outline as though encapsuled, clear refractile contents, in which is a smaller body of variable size analogous

¹ *Proc. Roy. Soc. Lond.*

² *Brit. Med. Journ.* 1890, ii. p. 1356.

to a nucleus (fig. 73). Occasionally the refractile contents present a radial striation or granulation.

These bodies are usually single, but may number as many as eight or ten, and sometimes they invade the epithelial nucleus. Sporulation has been described, but is very doubtful, and in the fresh tissues a contractile movement of the capsule has been stated to occur. The 'cancer parasites' are most numerous in rapidly growing cancers, and especially at the growing margin, and are said to be absent from normal tissues, granulomata, &c.

In stained preparations the nucleus is well coloured, but the refractile contents are usually only tinged. All attempts to cultivate this supposed organism have failed, and with a few doubtful exceptions inoculation experiments with cancer tissues have been unsuccessful. Other structures, such as the amœbiform bodies of Korotneff, have also been described as parasites in carcinoma, and in sarcoma somewhat similar appearances are said to have been noticed.

The molluscum bodies have also been regarded as parasitic (coccidial) in nature, but with them also inoculation and cultivation experiments have failed.

The chemistry of carcinoma and the molluscum bodies may be treated together. Glycogen is more or less abundantly present in most tumours. Proteoses were found to be absent by Shattock;¹ this the writer² confirmed, failing to find any in scirrhous carcinoma, spindle-celled sarcoma, and in fibro-adenoma. As the capsule of many protozoa seems to consist of chitin, Shattock and Brodie³ attempted to isolate this substance from malignant growths, but were unable to detect any trace. They likewise failed to detect any cellulose. A rough calculation of the possible amount of

¹ Morton Lecture on Cancer, *Brit. Med. Journ.* 1894, i. p. 1066.

² Report to the Scientific Grants Committee, *Brit. Med. Journ.* 1895, ii. p. 206.

³ Morton Lecture on Cancer, *loc. cit.*

chitin or cellulose present in malignant growths shows that these substances could hardly be detected by chemical methods.

Besides its staining reactions and the attempt to isolate chitin or cellulose, the writer has not come across any papers on the chemical reactions of the cancer parasite. As regards analogous organisms, Malassez and Delépine mention the blackening of the bioplasm of the *coccidium oviforme* with osmic acid and the brown staining with iodine (see p. 335).

Török and Tommasoli¹ have investigated chemically the nature of the molluscum bodies of molluscum contagiosum, and have compared them with the *coccidium oviforme*. The molluscum bodies are but slightly altered by prolonged treatment with such strong reagents as concentrated sulphuric, acetic, nitric, hydrochloric, and formic acids, caustic potash and ammonia, and by peptic digestion. The coccidia, on the other hand, are much more attacked. With acetic, sulphuric, and nitric acids the bioplasmic contents are gradually disintegrated and dissolved, and finally only the capsule remains, and caustic potash also profoundly alters them. Török and Tommasoli conclude from these experiments that, from their inertness, the molluscum bodies cannot be parasitic in nature.

Macallum² has also investigated the nature of the molluscum bodies. He, too, comes to the conclusion that these are not parasitic. He finds that although they give a brown colouration with iodine, the reaction is not an amyloid (lardaceous) one, for it is unchanged by dilute sulphuric acid. He concludes that they exhibit the reactions of keratin, or of eleidin, a substance convertible into keratin, both as regards their iodine reaction and also by containing iron.

¹ 'Ueber das Wesen Epithelioma Contagiosum,' L. Török und T. Tommasoli, *Monatsh. f. prakt. Dermatol.* x. 1890, p. 149.

² 'The Histology of Molluscum Contagiosum,' A. B. Macallum, *Journ. of Cutan. and Genito-Urinary Diseases*, March, 1892.

The writer¹ also has examined some of the chemical reactions of so-called cancer parasite and molluscum bodies with the following results:—

(a) *The Distribution of Phosphorus*.—For the detection of phosphorus the method of Lilienfeld and Monti² was made use of. Sections are soaked in a 20 per cent. solution of ammonium molybdate for from one to four days, washed in water containing a little ether, soaked for one-half hour to one hour in an ethereal solution of pyrogallie acid, and then mounted in balsam in the ordinary way. The depth of staining indicates the amount of organic phosphorus present. By this method no abnormal distribution of phosphorus was detected in the cells of carcinoma, sarcoma, or fibro-adenoma, the cell bioplasm being faintly, and the nuclei more deeply, stained, as is the case with cells generally. The nucleus of the cancer parasite stains to the same extent as other nuclei, but its bioplasm remains unstained. Molluscum bodies stain but faintly.

(b) *The Distribution of Iron*.—The detection of iron was carried out by Macallum's³ method. Sections are soaked in a mixture of equal parts of hydrochloric acid and potassium ferrocyanide of 0·5 and 1·5 per cent. strengths respectively. After washing in water they are mounted in balsam in the ordinary way; the depth of blue colour indicates the amount of iron present. No abnormal distribution of iron was observed in the cells of sarcoma, carcinoma, and fibro-adenoma. No iron could be detected in the cancer parasite.

(c) *Schultze's Solution* gave no trace of the blue cellulose reaction in the capsule of the cancer parasite or in the molluscum bodies.

(d) *Iodine and Osmic Acid* do not stain the bioplasm of the cancer parasite.

¹ Paper read before the Brit. Med. Assoc. 1895.

² *Zeitschr. f. Physiol. Chem.* xvii. 1893, p. 410.

³ *Journal of Physiology*, xvi. 1894, p. 272.

The so-called bioplasm of the cancer parasite, therefore, in its staining and chemical reactions resembles a watery fluid rather than bioplasm, and is possibly of the nature of vacuolation.

EXAMINATION.

1. *Russell's Bodies*.—Treat sections as follows :—

- (a) Wash in water.
- (b) Stain for ten to thirty minutes in a saturated solution of fuchsin in 2 per cent. aqueous carbolic.
- (c) Wash in water for a few minutes.
- (d) Absolute alcohol, half a minute.
- (e) One per cent. solution of iodine green in 2 per cent. aqueous carbolic, five minutes.
- (f) Dehydrate in absolute alcohol, clear in oil of cloves, and mount in balsam.

2. *The Protozoon Parasite*.—It cannot be said that the detection of the so-called protozoa in carcinoma is an aid in diagnosis. In order to demonstrate them a rapidly growing visceral carcinoma should be preferably chosen. Pieces from the growing edge are fixed in corrosive sublimate (p. 64, *d*) or Flemming's solution. *Paraffin* sections can then be stained with hæmatoxylin, using as a contrast eosin or rose bengale, or with the Ehrlich-Biondi reagent, or with anilin blue. (See Ruffer and Walker, 'Journ. Path. and Bact.' i. 1893, p. 395.)

VARIOLA AND VACCINIA.

The specific contagium of these two diseases (which, however, may be different manifestations of the same condition, as would be claimed by many) has not yet been discovered with certainty. A large number of observations have been made with vaccine lymph, but no distinctive bacterium has been obtained except by Klein and Copeman. Usually nothing has been isolated except the ordinary pyogenic organisms and many saprophytic forms. Le Dantec has isolated a streptococcus in variola, but this is probably a secondary infection. A bacillus first observed by Klein, and

subsequently more fully investigated by Copeman, has perhaps the most claim to be regarded as the specific organism in variola and vaccinia. It was first observed in vaccine vesicles at an early stage, but at maturation can no longer be found. It is a very fine bacillus, and these observers were unable to cultivate it. Subsequently Copeman found a similar organism in variola, and succeeded in cultivating the bacillus from both sources in eggs, and from such egg-cultures was able to inoculate calves. Recently Klein by storing variola lymph in 50 per cent. glycerin, and so getting rid of the saprophytic forms, has cultivated an organism which he terms the *bacillus albus variolæ*. Morphologically it closely resembles the bacillus observed in the lymph; it forms small, white, opaque, coherent, colonies on agar, but grows very feebly on gelatin. Involution forms occur, and it seems to belong to the group of diphtheria and xerosis bacilli. On inoculation it appeared to produce typical vaccinia.

The failure to isolate a bacterial form has induced many observers to seek for a parasitic protozoon in variola and vaccinia. L. Pfeiffer in 1887 observed roundish or ovoid bodies in the lymph in both diseases, which he regarded as sporozoa. Guarnieri found small bodies, about half the size of the nucleus, in the epithelial cells of the skin in the pre-pustular stage of variola. Small shining amœboid bodies were also noticed in the epithelial cells of the cornea of guinea-pigs inoculated with vaccine lymph. L. Pfeiffer confirmed Guarnieri's work, and also described these amœbiform parasites in the blood in variola and vaccinia, and of vaccinated calves. J. Clarke and Ruffer and Plimmer in this country described somewhat similar appearances. Ruffer and Plimmer¹ describe the supposed protozoon as a small round body, about 3 μ in diameter, lying within a clear vacuole in the bioplasm of the epithelial cell.

¹ *Brit. Med. Journ.* 1894, i. p. 1412.

Ogata has found bodies which he regards as parasitic protozoa and the causative agent of the disease in variolous and vaccine lymph. Reed likewise has found small granular amœboid bodies having a diameter of about one-third that of a red blood corpuscle, similar apparently to those described by L. Pfeiffer, in the blood of vaccinated children and monkeys, but has also observed them—and this is very important—occasionally in the normal blood of monkeys and children.

Ferroni and Massari state that appearances similar to those described by Guarnieri can be obtained in corneæ inflamed by croton oil or Indian ink, and therefore believe that the so-called parasites are derived from the nuclei or from emigrated leucocytes. Salmon considers that the so-called parasites in vaccinia and variola are more or less condensed balls of chromatin of extra-epithelial origin derived from the migratory, polynuclear leucocytes. So far the writer believes that the evidence in favour of the structures described as parasitic protozoa being really such is slight, and provisionally he would favour the bacillus of Klein and Copeman. If investigators would carefully study with high powers and various staining methods the structures present in normal blood and tissues, and compare them with those met with in disease—specific fevers, inflammations, &c.—probably many of the structures described as protozoa would no longer be regarded as such.

Literature on Variola and Vaccinia.

Practitioner, May 1896, pp. 459, 561 (Refs.)

Rep. Med. Officer, Loc. Gov. Board, 1896-97, p. 267.

Salmon, *Ann. de l'Inst. Pasteur*, xi. 1897, p. 289.

CHAPTER XX.

SOME DISEASES NOT PREVIOUSLY REFERRED TO—MICRO-ORGANISMS OF THE SKIN AND MUCOUS MEMBRANES.

BERI-BERI.

VARIOUS observers have attempted to cultivate a micro-organism in this disease. Pekelharing and Winkler have cultivated a coccus producing a white growth and resembling the *staphylococcus pyogenes albus* very closely. Hunter¹ obtained from two cases a similar coccus. He describes it as having a 'very active motion,' staining by Gram's method, producing white growths, and the earlier cultures liquefying gelatin slowly. It produced an acid reaction and formed indol. Some rabbits inoculated with it suffered from paresis, and the nerves were found to be degenerated.

CHANCRE, SOFT.

An extremely small bacillus, first described by Ducrey,² has been found in the ulcers and pustules. It has not been cultivated or inoculated successfully on animals, but can be inoculated, experimentally, from a chancre on to man. The bacillus does not stain by Gram's method. Buschke found the organism in all of twenty-one soft chancres examined by him; for staining he used a boric-acid methylene-blue solution.³

¹ *Lancet*, 1897, ii. p. 240 (Bibliog.).

² *Compte Rendu du Congrès Internat. de Dermatologie* (Paris, 1889), p. 229.

³ *Archiv. des Scien. Méd.* i. 1896, p. 146.

CONJUNCTIVITIS.

Axenfeld¹ states that conjunctivitis is usually of three kinds—viz. the acute, caused by the Koch-Weeks bacillus; the gonorrhœal; and the chronic, produced by a diplo-bacillus. The latter has some likeness to Friedländer's pneumococcus. It is decolourised by Gram's method, is aërobic, non-motile, and does not form spores, but slowly liquefies gelatin. Inoculation experiments gave positive results.

Morax and Beach² classify acute conjunctivitis as follows:—

(a) *Acute Contagious Conjunctivitis*, due to the Koch-Weeks bacillus. This organism is slender, occurs singly or in pairs, and both free and within the pus cells. It is decolourised by Gram's method. It is difficult to cultivate, and grows best on a serum-agar mixture, on which it forms small, punctiform, transparent colonies. It is non-pathogenic to animals, but in man sets up a typical acute conjunctivitis.

(b) *Gonorrhœal Conjunctivitis*, due to inoculation with the gonococcus.

(c) *Diphtheritic Conjunctivitis*.

(d) *Conjunctivitis of Streptococcic Origin*.

(e) *Conjunctivitis of Pneumococcic Origin*. Usually in children, and accompanied with coryza and scanty mucopurulent discharge.

Stephenson³ has also found the Koch-Weeks bacillus in cases of acute conjunctivitis. He considers the presence of this organism as diagnostic, and to detect it prepares two cover-glasses with the secretion, stains one with Löffler's blue, and the other by Gram's method with eosin. In the former the slender bacilli will be detected in large numbers, in the latter they will be decolourised.

¹ *Centr. f. Bakt.* xxi. 1897, p. 1.

² *Archives of Ophthalmol.* xxv. 1896, p. 54 (Bibliog.).

³ *Lancet*, 1897, i. p. 1531 (Bibliog.).

DIARRHŒA (SUMMER) OF INFANTS.

Booker¹ in an elaborate paper comes to the following conclusions: 'No single micro-organism is found to be the specific exciter of the summer diarrhœa of infants, but the affection is generally to be attributed to the activity of a number of varieties of bacteria, some of which belong to well-known species, and are of ordinary occurrence and wide distribution, the most important being a streptococcus and the *proteus vulgaris*. The streptococcus, termed *S. enteritis*, varies in morphology, and seems to be associated with two classes of cases, one of which simulates cholera, the other a typical enteric fever.

'Micrococci are present in all cases, sometimes in enormous numbers.

'When the diarrhœa passes into inflammation of the intestine and stomach, the condition remains no longer a local one, but becomes a general infection, and many bacteria can be cultivated from the organs, such as the colon bacillus, streptococcus, *proteus vulgaris*, and *bacillus pyocyaneus*.'

Lesage obtained a bacillus from the 'green diarrhœa' of infants which he believed to be the cause of this complaint. It is a small, motile, non-liquefying bacillus, producing on gelatin a whitish, expanded growth with crenated margins, and giving rise to a green fluorescence in the medium.

DISTEMPER.

Galli-Valerio² describes in distemper an oval bacillus 1.25 to 2.25 μ long, sometimes motile and forming spores. It grows well on the ordinary culture media. In gelatin the growth is white without liquefaction, and accompanied with the formation of gas.

¹ *Johns Hopkins Hosp. Rep.* vi. 1897, p. 159 (Bibliog.).

² *Centr. f. Bakt.* 1te Abt. xix. 1896, p. 694.

HERPES ZOSTER.

Pfeiffer observed bodies in the cells of the vesicles which he believed to be protozoa. Gilchrist,¹ however, regards these merely as altered nuclei.

FOOT AND MOUTH DISEASE.

Streptococci have been isolated from the vesicles in this disease, but are probably only the *S. pyogenes*. Bussenius and Siegels² have isolated a bacillus, but a German commission comprising Löffler and Abel³ stated that they were unable to prove its ætiological significance.

MEASLES.

Doehle and Behla described small flagellated bodies which they believed to be protozoa in this disease. Canon and Pielicke found small bacilli in the blood, which Tchaïkovsky confirmed. They are motile, do not stain by Gram's method, and can be cultivated on agar and serum, on which they form delicate colonies.

MALTA FEVER.⁴

Synonyms, Rock or Mediterranean Fever.

A micrococcus (*micrococcus Melitensis*), first described by Bruce,⁵ has been isolated from the spleen in this disease; ten times by Bruce, twice by Gipps, and thirteen times by

¹ Comparisons of Protozoa and Blastomycetes with the so-called Parasites found in Various Lesions in the Skin. *Johns Hopkins Hosp. Rep.* i. 1896, p. 291 (Bibliog.).

² *Centr. f. Bakt.* xxi. 1897, p. 478.

³ *Ibid.* xxiii. 1898, March.

⁴ See Hughes, *Med. Chirurg. Trans.* 79, 1896, p. 209 (Bibliog.).

⁵ *Practitioner*, 1887, p. 161, and 1888, p. 241.

Hughes. The micrococci are very minute, grow slowly on gelatin without liquefaction, and in broth give rise to a diffuse cloudiness, with a white deposit, and without film formation.

On agar it grows as minute transparent colonies, which first appear, when inoculated from the spleen, in ninety to 125 hours. In thirty-six hours more the colonies become amber-coloured, and later still, in four to five days, they become opaque, of a slightly orange colour, and round, with granular margins. At 37° C. the cultures retain their vitality for three months, and become distinctly orange in colour. The cocci are decolourised by Gram's method.

Inoculated into rabbits, mice, and guinea-pigs negative results are obtained, but in monkeys it gives rise to a febrile condition.

MASTOID DISEASE.

See Otitis Media.

MUMPS.

Mecray and Walsh¹ have isolated from the parotid and blood in some cases of mumps a coccus resembling that described by Laveran and Catrin. It occurs chiefly as a diplococcus, but also in large groups. The colonies form circular, white, shining points, with slow growth and gradual liquefaction. On potato a white growth occurs; on blood serum a plentiful cream-coloured growth; and in litmus-milk, production of acid with coagulation.

NOMA.

Schimmelbush described long bacilli (4 to 8 μ) in the necrotic tissues in this disease, and Lingard and Klein have observed motile vibrios.

¹ *Med. Record* (N. Y.) 1. 1896, p. 440 (Bibliog.).

Bishop and Ryan ¹ in two out of three cases isolated an organism which culturally and morphologically resembled the diphtheria bacillus, but which only produced some local inflammation on inoculation into guinea-pigs. In the third case the *staphylococcus pyogenes aureus* and the *streptococcus pyogenes* were isolated. Guizzetti ² and Freymuth and Petruschky ³ have also isolated the Klebs-Löffler bacillus in noma.

OTITIS MEDIA.

The *diplococcus pneumoniae* is perhaps the commonest organism met with; next in frequency comes the *streptococcus pyogenes*, and then the pyogenic cocci. In scarlatinal otitis media, Blaxall ⁴ found the *streptococcus pyogenes* to be always present, and generally accompanied by other organisms, pyogenic cocci, &c. In thirty-seven cases of mastoid disease Blake ⁵ found the following organisms, and remarks that as a rule the same were found in the middle ear:—

Streptococcus	12
Staphylococcus	5
Diplococcus (? <i>pneumoniae</i>)	6
Streptococcus and diplococcus	5
Streptococcus and <i>bacillus fetidus</i> (? colon bacillus)	3
Streptococcus and <i>bacillus pyocyaneus</i>	1
Streptococcus and diplococcus	1
Streptococcus, staphylococcus, and diplococcus	2

In two of the cases no organisms were isolated.

OZÆNA.

Löwenberg has described in this disease encapsuled bacilli somewhat resembling morphologically the pneumo-bacillus. Some Italian observers have found bacilli apparently

¹ *Journ. Amer. Med. Assoc.* (Chicago), xxv. 1895, p. 1043 (Bibliog.).

² *Il Policlinico*, iii. M. (Fasc. 9) 1896, p. 405, *cl seq.* (Bibliog.)

³ *Deutsch. Med. Woch.* 1898, April 14, p. 332.

⁴ *Brit. Med. Journ.* 1894, ii. p. 113 (Bibliog.).

⁵ *Brit. Med. Journ.* 1897, ii. p. 1555.

identical with the diphtheria bacillus. Abel¹ also describes a bacillus resembling the pneumo-bacillus somewhat. It is this organism which produces the atrophy of the mucous membrane, but the fetor is due to the decomposition of the secretions by other organisms.

PEMPHIGUS.

A diplococcus has been isolated in acute pemphigus by Demme, and in the chronic form by Dähnhardt. Bulloch² and Russell Wells,³ in this country, seem to have isolated an identical organism, and the following description of it is taken from their papers. Cocci 0·8 to 1·5 μ in diameter, mostly arranged as diplococci, and staining by Gram's method. On surface agar the organism forms a thick, white, shining growth. In stab agar the growth has a 'nail-shaped' appearance. The colonies on agar are at first round, but later, in seven days, they throw out lateral projections and assume a rosette appearance. On gelatin the growth is slow and slight, with some, but not marked, liquefaction. On blood serum the growth resembles that on agar. On potato a whitish, semi-transparent, film forms. Milk is curdled. In broth it causes a general turbidity, with a whitish sediment, and sometimes a pellicle, which soon sinks. Guinea-pigs and mice inoculated or vaccinated with the organism died in four to eight days, fine hæmorrhages occurring in the lungs, and the cocci being obtained from the blood. No bullæ appeared on the skin.

PERITONITIS.

Treves⁴ classifies peritonitis as follows:—

(a) Due to infection from within, as in strangulation and

¹ *Zeitschr. f. Hyg.* xxi. p. 89.

² *Brit. Jour. Dermatol.* viii. 1895, Nos. 91, 92, p. 159 (Bibliogr.).

³ *Lancet*, 1896, i. p. 1219 (Bibliogr.).

⁴ The Lettsomian Lectures on Peritonitis, *Brit. Med. Journ.* 1894, i. p. 229, *et seq.* (Full Refs.).

perforation of the bowel, appendicitis, and in suppurative conditions of the gall bladder and biliary canals; organism present, the colon bacillus.

(b) Due to infection from without, as in puerperal peritonitis, and that following wounds and operations; organisms present, the pyogenic cocci, and especially the *streptococcus pyogenes*.

(c) Peritonitis due to the pneumococcus.

(d) Peritonitis due to the tubercle bacillus, tubercular peritonitis.

(e) Peritonitis met with in rheumatism, gonorrhœa, syphilis, Bright's disease, &c., not yet associated with any specific organism.

(f) Chemical peritonitis (α) from within, possibly due to the absorption of chemical products from the intestine, for in certain cases the exudation has been found to be sterile; (β) following the injection of certain chemical irritants.

Cases of Peritonitis in Man in which Micro-organisms were found in the Exudation.

	Frinkel	Tavel and Tanz	
	Found alone	Found alone	Found in association
<i>Bacillus coli communis</i>	11	15	16
<i>Streptococcus</i>	7	3	15
<i>Staphylococcus</i>	1	2	6
<i>Pneumococcus</i>	1	0	2
	20	20	39

PERTUSSIS.

Afanassjew observed large numbers of bacilli in the sputum in this disease. Koplik, by sowing the pellets on

solidified hydrocele fluid, obtained a pure culture of a small and delicate bacillus measuring 0·8 to 1·7 μ in length. It forms a white growth, does not produce spores, and stains with methylene blue like the diphtheria bacillus, clubbed involution forms also occurring. It is pathogenic for mice, but not for guinea-pigs. It was isolated in thirteen out of sixteen cases.¹

PSILOSIS OR SPRUE.

Thin² considers this disease to be due to an abnormal fermentation in the intestine brought about by some organism which has not yet been isolated.

RHEUMATISM (ACUTE).

Singer³ in ninety-two cases isolated staphylococci and streptococci, and considers these organisms to be of ætiological importance, and regards the disease as pyæmic in nature.

Achalme⁴ describes a bacillus isolated by him in twelve cases of acute articular rheumatism. The bacillus is a large rod not unlike that of anthrax, with which it has perhaps been confounded (cases of aortitis, Oliver ; myelitis, Baumgarten, &c.). In culture media it forms filaments of variable length. It is strictly anaërobic, slightly motile in young cultures, and forms terminal spores. It stains well by the ordinary anilin dyes and also by the methods of Weigert and Gram. It can be cultivated, anaërobically, in the ordinary culture media, giving whitish growths with the formation of gas. Milk is coagulated. It grows well between 30° and 35° C., but not below 25° C. or above 40° C. It liquefies gelatin, produces acids, and ferments saccharose without inverting it. Inocu-

¹ *Brit. Med. Journ.* 1897, ii. p. 1051 (Bibliog.).

² *Psilosis or Sprue*, J. & A. Churchill, 1897.

³ *Berl. klin. Woch.* 1897, No. 31.

⁴ *Ann. de l'Inst. Pasteur*, xi. 1897, p. 815.

lated into animals it produces dilatation of the arterioles and thrombosis, negative chemotaxis, a serous effusion into the cellular tissues, and congestion of and hæmorrhages into the serous membranes and viscera. It is pathogenic for the guinea-pig, mouse, and rabbit, but not for the dog.

RHEUMATOID ARTHRITIS (ARTHRITIS DEFORMANS).

Schuller described a small bacillus in this disease.¹ Blaxall² found in the synovial fluid, and occasionally in the blood, a minute bacillus measuring 2μ in length. It possesses marked polar staining, is decolourised by Gram's method, and can only be stained by prolonged immersion in the solution. Blaxall obtained the best results by fixing cover-glass specimens by passing six times through the flame, treating with dilute acetic acid for two minutes, washing and drying, then staining in anilin methylene blue for three to five days, washing in running water for some hours, rinsing in distilled water, drying and mounting. The organism can be cultivated on agar and serum and in broth. In a clear broth, after three days, minute shining, yellowish particles appear and increase in amount, giving rise on shaking the flask to an appearance of 'gold dust.' On agar and serum an extremely delicate film of growth appears, only visible with a lens. In cultivation the bacillus is found to be non-motile, to form zooglœa masses, and sometimes to grow into longer individuals. It does not grow on gelatin. Inoculation experiments on animals failed.

RHINOSCLEROMA.

Bacilli have been described by Von Frisch in this disease. They are short, with rounded ends, encapsuled, and frequently

¹ *Berlin. klin. Woch.* Sept. 4, 1893.

² *Lancet*, 1896, i. p. 1120 (Bibliog.)

linked in pairs. It is non-motile, does not stain by Gram's method, and forms on gelatin a whitish growth without liquefaction like that of Friedländer's pneumo-bacillus.

SYPHILIS.

Small bacilli were observed by Lustgarten in syphilitic lesions and ulcers. In size and appearance they resemble the tubercle bacillus; they differ from it, however, in staining reaction. They occur in rounded cells, usually one or two together, occasionally more numerous. They have not been cultivated. They can be demonstrated by staining sections in anilin gentian violet for twelve to twenty-four hours, washing in alcohol, decolourising in a 1·5 per cent. solution of potassium permanganate for ten seconds, rinsing in a 5 per cent. solution of sulphuric acid, and then in water; and if not decolourised, repeating the potassium permanganate, &c., then dehydrating, clearing, and mounting. It has been suggested that this syphilis bacillus is the smegma bacillus, but the two seem to have a different staining reaction (see *Smegma Bacillus*).

Eve and Lingard also described a bacillus in syphilis, but it is generally regarded as a saprophytic form. Recently protozoon parasites have been described in syphilis.

Niessen¹ has lately described a strepto-bacillus in this disease.

TRACHOMA.

Sattler described a diplococcus in this disease. It is strictly aërobic, forms a scanty, whitish growth on gelatin, which is not liquefied, and a copious, viscid, white growth on agar and serum.²

¹ *Centr. f. Bakt.* xxiii. 1898, p. 49.

² See Lawson, *Roy. Lond. Ophthalm. Hosp. Reps.* xiv. 1897, p. 484 (Bibliog.)

TYPHUS FEVER.

Levaschew¹ states that he has observed in the blood of patients suffering from typhus fever round, highly refractile bodies, the poles of which are occasionally elongated so as to form a flagellum on either side. The organism can be cultivated by smearing droplets of the blood on agar-tubes and incubating at 37° C. In gelatin stab-cultures liquefaction occurs. The organism is strictly aërobie, and inoculation experiments failed. McWeeney² obtained negative results by culture methods.

YELLOW FEVER.

The *bacillus icteroides* or bacillus of yellow fever, isolated by Sanarelli,³ has at first sight no morphological characteristics. It is a small bacillus with rounded extremities, for the most part united in pairs in cultures, and forming small groups in the tissues, 2 μ to 4 μ in length, and its breadth is as a rule two or three times less. It is very pleomorphous. Examination of the tissues fails to give good results excepting in cases where the patient dies without secondary septicæmia. Even in cases bacteriologically examined and yielding the purest results, the bacillus is not easily seen in the tissue sections because their number is often very small. Still, by employing suitable methods it can be found in the organs, usually united in little groups, always situated in the small capillaries of the liver, kidney, &c. The best means of demonstration is to place a fragment of liver, obtained from the body whilst fresh, in the incubator at 37° C. for twelve hours. This encourages the multiplication of the specific microbe.

The bacillus develops well on all the ordinary media. In

¹ *Arch. des Sc. Biolog. de l'Inst. Impér. de Med. Exper. de St. Petersbourg*, iv. 1896, No. 4.

² *Brit. Med. Jour.* 1898, i. p. 881 (Bibliog.).

³ *Ibid.* 1897, ii. p. 7.

gelatin plate cultures it forms roundish colonies, transparent and granular. The granulation of the colony becomes more marked, and usually a central or peripheral nucleus, completely opaque, becomes apparent. Finally, the whole colony becomes opaque, but does not liquefy gelatin. In streak-cultures on gelatin, brilliant little opaque drops are formed like drops of milk. In meat broth it develops slowly without forming a pellicle or flocculent deposit. On solidified blood serum the growth is almost invisible.

In cultures on agar-agar, if grown at a temperature of 20° C. to 22° C., the colonies are like drops of milk, opaque, prominent, and with pearly reflections. If, however, the cultures are placed in the incubator for from twelve to sixteen hours, and afterwards kept in a room for a like period, the colonies show a flat, central nucleus, transparent and bluish, surrounded by a prominent and opaque zone, the whole resembling a drop of sealing-wax. This characteristic appearance can be obtained in twenty-four hours, and is valuable, as it establishes the bacteriological diagnosis of the *bacillus icteroides*. It is facultative anaërobic, stains by Gram's method, slowly ferments lactose, more actively glucose and saccharose, but does not coagulate milk; it is very resistant to drying, is killed in water at 60° C., and also by the solar rays in seven hours, but lives in sea-water for a long time.

Sternberg¹ claims that the *bacillus icteroides* of Sanarelli is identical with the *bacillus X* isolated by him in cases of yellow fever in Havana in 1889.

MICRO-ORGANISMS OF THE SKIN AND MUCOUS MEMBRANES.

Skin.—A very large number of micro-organisms are always present on the skin of every part of the body, and it is impossible to touch the smallest point without obtaining a culture if an

¹ *Centr. f. Bakt.* 1te Abt. xxii. p. 145.

inoculation be made. The pyogenic cocci are everywhere present, a variety of the *S. pyogenes albus*, the *S. epidermidis albus* of Welch, being perhaps the commonest. Sarcinæ, bacilli, and moulds are also present. On the skin of the groin, scrotum, and vulva the smegma bacillus occurs. From sweating feet various organisms have been isolated, which on culture evolve a disagreeable odour, among which is the *bacterium fetidum* of Thin.

Conjunctivæ.—Some observers have stated that the conjunctiva is generally sterile. A certain number of organisms are, however, usually present, though they are not numerous, and if artificially inoculated, the excess is rapidly got rid of.

Randolph¹ states that the normal conjunctiva always contains organisms, the commonest species being the *staphylococcus epidermidis albus* of Welch, a coccus resembling the *staphylococcus pyogenes albus*, but growing, liquefying gelatin, and coagulating milk, more slowly.

Nose.—In the anterior nares, crusts and vibrissæ micro-organisms are present in great abundance, but contrary to the usual opinion, St. Clair Thomson and the writer² showed that the mucous membrane of the interior of the nose is comparatively sterile, and when organisms are present they are very scanty. Moreover, organisms artificially deposited were found to be rapidly disposed of. After two hours, for example, *prodigiosus* inoculated on to the inferior turbinate could not be detected by cultivation. Wurtz and Lermoyez asserted that the nasal mucus is germicidal, but St. Clair Thomson and the writer³ were unable to confirm this, though it may have an inhibitory action.

Air Passages.—Below the larynx under normal conditions the air passages are free from micro-organisms. Expired air is also free from organisms, and the air from the naso-pharynx after passing through the nasal cavities is deprived of the majority of its organisms.⁴

Mouth.—Micro-organisms of all kinds are present in the

¹ *Archives of Ophthalmol.* xxvi. 1897, p. 379.

² *Medico-Chirurg. Trans.* 78, 1895 (Bibliog.).

³ 'The Fate of Micro-organisms in Inspired Air,' *Lancet*, 1896, Jan. 11 (Refs.).

⁴ *Ibid.*

buccal cavity in the greatest abundance—leptothrix, bacilli, pyogenic cocci, sarcinæ, and spirilla are almost always to be found. The *streptococcus pyogenes*, *staphylococcus pyogenes aureus*, and *diplococcus pneumoniae* are frequently present. Certain organisms have their normal habitat in the mouth, are difficult to cultivate, and are of considerable importance in the production of dental caries. The normal saliva is germicidal to some extent.

Stomach and Intestine.—Although a vast number of organisms gain access to the stomach, a large number are destroyed by the acid gastric juice. At the same time, a considerable proportion are able to survive—sarcinæ, and lactic and butyric acid bacilli. In the intestine multitudes of organisms find a suitable nidus, of which non-sporing bacilli predominate. Anaërobic sporing forms also occur.

Urinary and Genital Organs.—The meatus urinarius and distal portion of the urethra contain a certain number of organisms, which increase in number in inflammatory conditions. (See p. 148.) The deeper portion of the urethra, however, is free from organisms, and the bladder is sterile. The genital tract in the female up to the middle zone of the cervix contains organisms, but the uterus and fallopian tubes are normally sterile.

CHAPTER XXI.

THE BACTERIOLOGY OF WATER, AIR, AND SOIL, AND THEIR
BACTERIOLOGICAL EXAMINATION—SEWAGE—BACTERIOLOGY
OF MILK AND FOODS.

Some of the Commoner Organisms found in the Air, Water, and Soil.

THE BACTERIOLOGICAL EXAMINATION OF WATER.

THE bacteriology of water has now assumed considerable importance, and its examination by bacteriological methods has become a routine part in analysis for hygienic purposes.

The bacterial flora of natural waters is a very varied one. In surface waters, such as streams, ponds, and shallow wells, the organisms met with are largely derived from the air and soil through which the water has passed. When uncontaminated from human or animal sources, by the air of towns, sewage, manure, &c. they consist of bacilli, the majority of which are chromogenic, non-liquefying, and develop on culture media at a temperature of 22° C. or thereabouts only, not at blood-heat; also of some sarcinæ and a few micrococci. When, however, the water passes through cultivated lands, or receives sewage, the number of organisms is enormously increased; a large proportion of them liquefy gelatin and develop at blood-heat, while members of the colon group appear more or less numerously. Whereas water from shallow wells has a bacterial content nearly as great as the surrounding surface water, that from deep wells, especially in the chalk, is remarkably free from organisms. The following

table illustrates the number of organisms which may be met with in water from different sources :—

Source.	Number of Organisms per Cubic Centimetre.
Freshly fallen snow	34-38
Ice	(very variable) 30-1,700
Rain water (Paris)	4-5
Rhone, above Lyons	75
Rhone, below Lyons	800
Rhine, at Mühlheim	average about 20,000
Thames, at Hampton (Frankland)	(variable) 2,000-90,000
Deep well in the chalk (Frank- land)	7-21
Surface well	1,200
Spring water, Reigate (Frank- land)	8
Lake of Lucerne	8-50
Loch Katrine (Frankland)	74
Filtered water supplied to London (Frankland)	average not more than 100
Sewage (Frankland)	26,000,000

The number of bacteria in water varies considerably with its source, at different seasons, and under different climatic conditions. Sand filtration is capable of removing over 90 per cent. of the organisms originally present, but only when the surface scum on the sand has formed and is undisturbed; a new filter bed, or one recently cleaned out, may allow a large proportion of the organisms to pass through.

Simple storage in a reservoir also effects a considerable diminution in the number of bacteria contained in the water owing to subsidence.

The following tables illustrate these points :—

Variation of Number of Bacteria at different Seasons
(Number in 1 c.c.)

Thames Water at Hampton. (Frankland.)	River Lee Water at Chingford. (Frankland.)
December . . . 10,600	January . . . 31,000
March . . . 66,000	April . . . 84,000
June . . . 3,500	July . . . 2,190
September . . . 1,740	October . . . 2,310

Removal of Organisms by Sand Filtration (Number
in 1 c.c.). *Frankland.*

	January.	April.	July.	October.
Thames water unfiltered (Hampton) . . .	45,000	12,250	3,000	8,600
Chelsea Water Co. . .	159	94	59	34
Southwark Water Co. . .	2,270	77	380	61

Diminution of Organisms by Storage (Number in 1 c.c.).
Frankland.

Thames water unfiltered (Hampton)	. . .	1,437
After passing through one storage reservoir	} before filtration	318
After passing through two storage reservoirs		
		177

According to Frankland the following factors influence the number of organisms present in the water distributed by a water company:—

1. *Storage Capacity for Unfiltered Water.*—A large storage capacity permits a selection to be made when to draw the water from the supply, so as to avoid taking it when this is in a bad condition, as in flood time or drought. Moreover, storage alone markedly diminishes the number of organisms, partly by subsidence, and partly probably owing to the struggle for existence going on among them.

2. *Thickness of Fine Sand in the Filter Beds.*—It is the

fine sand only which has to be taken into account in estimating the removal of organisms and efficacy of a filter bacteriologically. It should probably form a layer not less than 3 feet to 3 feet 6 inches in thickness.

3. *The Rate of Filtration.*—The removal of organisms is less perfect when the rate of filtration is increased. It should not exceed about 1.5 gallons per square foot per hour.

4. *The Renewal of the Filter Beds.*—The filter beds after cleaning allow a greater number of organisms to pass through. The normal efficiency seems to be rapidly regained, within two or three days, while the efficiency of the filter beds (bacteriologically) does not appear to be reduced by prolonged use.

Besides storage and filtration, sedimentation, in the presence of fine particles, also effects a very marked removal of micro-organisms from water. In Clark's process for the softening of waters possessing a considerable temporary hardness from the presence of soluble bicarbonate of lime, a suitable proportion of lime-water is added, which results in the precipitation of the lime in the added lime-water together with the lime present in the water as bicarbonate, in the form of a fine precipitate of calcium carbonate with which the organisms are carried down.

The bacteriological analysis of water may afford valuable indications as to the purity or otherwise of a water; it cannot, however, supplant in any way the chemical analysis; the two methods supplement each other and should be worked side by side. The search for pathogenic organisms, though most important, is not everything, for not only are they extremely difficult to find, and a negative result therefore is of little value, but the source of infection may have ceased and the pathogenic organisms have disappeared before the water is submitted for examination, as will be referred to when dealing with the search for the comma bacillus and *B. typhosus*. The number and character of the organisms must also be taken

into account, though at the same time caution is required in interpreting the results, for it should be clearly borne in mind that waters differ bacteriologically just as they do chemically, and that to reject a water because it contained rather more than the recognised number of organisms would be equivalent to condemning it, say, for an excess of chlorine, the strata and geological features of the district being unknown. Strictly speaking, the mean bacterial content of a water or supply should be ascertained, and then any departures from this mean may yield valuable information. The full value of a bacteriological examination, for example, would be seen if systematic analyses under similar conditions were made of the supply from the filter beds or of the supply as it leaves the water company's premises. In a year or so a standard of the average number of bacteria normally present in the water at different seasons would be arrived at, and by continuing the examinations at regular intervals, any sudden and marked departure from the standard so ascertained would indicate that something was wrong with the filter beds or elsewhere, and would indicate the necessity for an investigation.

THE BACTERIOLOGICAL EXAMINATION OF WATER.

The specimen of water should be collected in clean bottles, sterilised preferably by heat. If, however, the bottles be thoroughly cleaned and rinsed out with a little strong sulphuric acid, and then thoroughly rinsed several times with the water to be examined before taking the specimen, no error will be introduced. In taking the specimen the following points must be attended to :—

1. If taken from a tap, the water should be allowed to flow for at least five minutes before the specimen is collected.

2. The water from a cistern is not a fair sample of the water supply ; to be so the specimen should be taken direct from the mains.

3. If taken from a stream or pond, the bottle should be held about a foot below the surface before the stopper is removed.

4. If taken from a well, the conditions should be noted, *i.e.* whether the well has been recently disturbed or no, whether the pumps have been in operation, &c., for such may markedly influence the number of bacteria found.

The specimen should then be examined with as little delay as possible, for if allowed to stand for any time a large increase in the number of bacteria may take place. Frankland, for example, found that in distilled water, even at the ordinary temperature, organisms multiplied enormously : —

Hours	Number of Organisms in 1 c.c.
0	1,073
6	6,028
24	7,262
48	48,100

Similar observations have been made with natural waters, and it is essential, therefore, if reliable results are to be obtained, to examine the specimen at once. If this cannot be done the specimen should be packed in ice; the cold will then inhibit multiplication to any extent.

In spring water the organisms are found to multiply much more rapidly during the first few days, after which time they become less and less numerous; but in very impure water multiplication is slow, and the number much more persistent.

The routine bacteriological examination of the specimen falls under the following sections :—

1. The number of organisms present in a given volume.
2. The ratio of organisms liquefying gelatin to those which do not.
3. The number of organisms present in a given volume which will develop at blood-heat.
4. The search for the colon bacillus or other pathogenic species.

5. The virulence of a peptone-water culture.

1. *The Number of Organisms present.*—The number of organisms present in a given volume, 1 c.c. being that usually adopted, is estimated by means of gelatin plate cultivations. The gelatin for this purpose should be as nearly neutral as possible, but any tendency to acidity must be avoided; it is preferable to have it somewhat alkaline rather than the least acid. The gelatin tubes are melted in the ordinary way and inoculated with a measured volume of water. The specimen is well shaken up and the water measured by means of a sterilised pipette of 1 c.c. capacity, graduated in hundredths. An assistant holds the tubes and withdraws the wool-plugs, and the required volume is then run into each. For an ordinary drinking-water four tubes may be inoculated with 0.5, 0.2, 0.2 and 0.1 c.c. respectively (making in all 1 c.c.), and a duplicate set should also be prepared. The contents of the tubes are thoroughly mixed and then poured into sterilised Petri dishes. The eight dishes are incubated at 22° C., and should be examined daily, both morning and evening. If possible the plates should be incubated for five to seven days, but in many instances, owing to the number of liquefying forms, it may be necessary to enumerate the colonies on the third or even the second day. The colonies which develop in each plate are counted, and their number represents (roughly) the number of organisms present in the specimen. The results are reduced to number of organisms per cubic centimetre. If the numbers in each plate, allowing for the difference of volume of water added, correspond fairly well, the number per cubic centimetre is found by adding together the number of colonies on each plate and taking the mean of the two sets. If, however, the numbers do not correspond very well, it is probably more accurate to reduce each number to the number per cubic centimetre, add the results together, and obtain the mean per cubic centimetre by dividing by four. As a certain number of organ-

isms remain in the last drops of gelatin in the tubes, these may be kept and incubated, and the number of colonies which develop added to the number obtained on the plates.

If there is any suspicion attaching to the water, one set of plates is made with the undiluted water as described above, but the other set should be made with the water diluted ten or a hundred times with sterile water, for in the plates made with the undiluted specimen the colonies may be so numerous as to be uncountable.

In order to count the colonies ink lines may be drawn across the bottom of the Petri dishes so as to divide them into sectors. The colonies in the sectors are then much more easily counted; or if the colonies be very numerous and evenly distributed, the number in two or three of the sectors may be counted, and the total number on the plate estimated by calculation.

As already indicated, no definite conclusions can be drawn from the number of organisms present unless the source of the water and the conditions under which the sample was taken are accurately known. Koch laid down as a standard that a good water should not contain more than 100 organisms per cubic centimetre; but under ordinary conditions waters rarely come up to this standard, and anything less than 500 organisms per cubic centimetre may be regarded as fairly good.

2. *The Ratio of Organisms liquefying Gelatin.*—This is readily ascertained from the gelatin plates employed to enumerate the organisms. It is stated that the ratio of liquefying to non-liquefying forms should not exceed one to ten. The normal bacteria of water are largely non-liquefying, but if the water be contaminated with sewage the number of liquefying organisms becomes largely increased.

3. *The Number of Organisms which will develop at Blood-heat.*—The majority of the normal bacteria of water do not develop at blood-heat, whereas those derived from sewage to

a large extent do. Hence, if by the gelatin plate method a large number of organisms are found to develop at 22° C., and a large proportion of them do not develop at blood-heat, the water would be regarded as of much better quality than if this were not the case. In order to estimate the number of organisms which develop at blood-heat, agar plates are prepared with about 0·5 c.c. of the water, and incubated in the inverted position at 37° C. for twenty-four hours, and the colonies are then counted. The following table, taken from a paper by Pearmain and Moor,¹ illustrates the number of organisms which develop at blood-heat in pure and impure water respectively:—

	Polluted Surface Well-water			Water of Average Quality	
	(a)	(b)	(c)	(d)	(e)
Approximate number of organisms per cubic centimetres in the original water, as determined by a gelatin-plate culture	800	1,050	1,400	180	270
Number of organisms per cubic centimetres appearing on an agar-agar plate culture, after incubating at blood-heat for twenty-four hours	220	180	350	10	5
Approx. number of organisms per cubic centim. determined by an agar-agar plate culture, after incubating the water at blood-heat	800,000	uncountable	uncountable		

4. *The Search for the Colon Bacillus and other Pathogenic Species.*—The search for pathogenic organisms, especially the typhoid bacillus, is a difficult matter, and need not be undertaken unless specially required. The colon bacillus, however, should always be examined for by Parietti's method. As the colon bacillus is such an ubiquitous organism some care must be exercised in condemning a water because of its presence; even the purest waters may contain it in small numbers. If the colon bacillus is met with in large quanti-

¹ *Journ. of the Soc. of Public Analysts*, 1896.

ties it is, to say the least, very suspicious; if any number of colonies are met with in the gelatin plates used for enumeration the water should be condemned; if it is met with in *all* the Parietti tubes it is highly suspicious, but if in only one or two, and those containing the larger amounts of water, it is of less importance. Klein describes a form of *proteus*, which occurs in sewage and sewage-contaminated water, and somewhat resembles the colon bacillus in its growth in gelatin and by developing in carbol-gelatin and Parietti broth. It differs from the colon bacillus in being a well-marked rod, and in not curdling milk or producing gas.

Needless to say the presence of the typhoid bacillus or comma bacillus is sufficient to condemn a water.

Below are given the methods for the isolation of the typhoid bacillus, colon bacillus, and the comma bacillus of cholera.

TYPHOID BACILLUS.

The isolation of the typhoid bacillus from water is fraught with considerable difficulty, and although the polluted water which has been proved to be the source of infection in many well-marked epidemics has been submitted to expert bacteriological examination, in a few instances only has the typhoid bacillus been isolated with certainty. The difficulties are two-fold. The incubation period of enteric fever being something like two to three weeks, it is evident that widespread infection may have taken place from a temporary source of pollution, and the water supply have subsequently regained its normal character before the occurrence of the epidemic, which alone signalises the said pollution. In the next place, supposing that the pollution has continued up to the time of the epidemic, we can examine by bacteriological methods small quantities of water only, and unless the typhoid bacilli are uniformly distributed and numerous they are very apt to be missed. This difficulty is enhanced by the fact that, although we are able to inhibit the growth of most of the organisms accompanying the typhoid, the colon bacillus in all cases flourishes luxuriantly; and as it is likely to be present

in considerable numbers, especially where the source of pollution is from sewage, the task of isolating a few typhoid bacilli from a large admixture of the colon bacillus becomes a very considerable one. It must be clearly understood, therefore, that all methods for the isolation of the typhoid bacillus, with perhaps one exception, are methods for the isolation of the typhoid bacillus *plus* the colon bacillus; and as the colon bacillus is the more resistant form, if the inhibitory chemical agent, which usually forms the basis of these methods, be added in excess it may result in the suppression of the typhoid and isolation of the colon bacillus alone.

In order to examine a large volume of water for the typhoid bacillus, Klein recommends passing 1000–1200 c.c. of the water through a sterile Berkefeld, removing the deposit of organisms on the filter by brushing with a sterile brush, and adding the brushings to the Parietti tubes or Elsner medium.

A. INHIBITION BY MEANS OF PHENOL.—The typhoid and colon bacilli are among the few organisms which will develop in the presence of small quantities of acid and of phenol.

(a) *Carbolised Gelatin*.—Ordinary nutrient gelatin with the addition of 0.05 per cent. of phenol. Plate cultivations are made in the usual way, taking 0.1 to 1 c.c. of the water for each plate and incubating for forty-eight hours at 22° C.

(b) *Parietti's Method*.—This depends on the combined use of an acid and phenol. The following solution is prepared:—

Phenol	5 grams
Hydrochloric acid	4 „
Distilled water	100 c.c.

A series of test-tubes, say a dozen, each containing 10 c.c. of sterile nutrient broth, are taken, and 0.1 to 0.3 c.c. of this solution added to each, four sets of three tubes each, containing respectively 0.1, 0.2, and 0.3 c.c. of the Parietti solution being usually made. To each tube is then added from 1 c.c. to 3 c.c. of the suspected water, and they are incubated at 37° C. for twenty-four hours. Those tubes which show a growth in this time will probably contain the colon or typhoid bacillus, or a mixture of these (the colon bacillus largely predominating), and nothing else. The typhoid bacillus will probably not be found in the

tubes containing 0·3 of the Parietti solution. Plate cultures of ordinary gelatin, or, better, Elsner's medium (see below) are then prepared, incubated for forty-eight hours at 22° C., and examined.

B. ELSNER'S MEDIUM.¹—This is prepared as follows: Peel potatoes and remove eyes. Reduce them to a paste by grating on a large nutmeg-grater. Weigh out 500 grams of this paste and add to it one litre of tap-water, and allow the mixture to stand for twelve hours in a cool place, and then strain through muslin. Add to this litre of potato broth 150 grams of gelatin and steam for half an hour. This mixture is strongly acid, and the acidity must be reduced by the addition of deci-normal caustic soda solution until the reaction is only faintly acid. Allow it to cool to 60° C., and add the white of an egg. Steam again for half an hour, filter through paper in a hot-water funnel, and introduce into small Erlenmeyer flasks, 50 c.c. in each. Sterilise in the steamer for twenty minutes on two successive days.

Just before use melt and add 1 per cent. of potassium iodide (*i.e.* $\frac{1}{2}$ gram to each flask), and dissolve. The gelatin medium may then be divided into about six equal portions by pouring into half a dozen sterilised test-tubes.

For the isolation of the typhoid bacillus from the stools several sets of plates may be made, inoculating the first tubes of each set with 1 to 6 loopfuls of the faecal matter. Water may be examined either by making sets of plates from Parietti tubes, or by making plates directly with the water, using 1 to 3 c.c. for each plate.

On the Elsner medium the typhoid bacillus forms characteristic colonies. After forty-eight hours' incubation they are still so minute as to require a low power of the microscope to see them. They then appear as circular colonies, almost structureless, pale, and like small droplets of water, while the colonies of the colon bacillus are larger, more granular, and browner in colour. The acidity of the medium prevents the development of most forms of bacteria met with in water or faeces. This method certainly seems to give the best results for the isolation of the typhoid bacillus from faeces. Sterling,² for example, by the old

¹ *Zeitschr. f. Hyg.* xxi. 1896, p. 25.

² *Centr. f. Bact.* xxii. Abt. I. 1897, p. 334.

methods isolated it 17 times only in 216 examinations, or 8 per cent., whereas by Elsner's method he found it 41 times in 63 examinations, or 66 per cent., and in the second and third week of the disease 27 times in 31 examinations, or 87 per cent.

Stoddart¹ has also devised a method, based on the fact that in a medium which is soft the typhoid bacillus spreads throughout it, while the colon bacillus retains its usual discrete colonies. A special gelatin agar mixture is employed, and incubated at 35° C.

THE COLON BACILLUS.

The colon bacillus may be readily isolated by some of the methods employed for the typhoid, viz. by carbolised gelatin plates or by Parietti's method and subsequent sub-culturing. By the following method much larger quantities of water can be dealt with.

*Abba's Method.*²—The following solution is prepared :—

Lactose	200	grams
Peptone	100	"
Sodium chloride	50	"
Water	1000	c.c.

This is heated in the steam steriliser for half an hour, then filtered, and again sterilised. A litre of the suspected water is taken and 100 c.c. of this solution added to it, together with 0.5 c.c. of a 1 per cent. alcoholic solution of phenolphthalein and sufficient (about 2 to 3 c.c.) of a cold saturated solution of sodium carbonate to render the mixture of a permanent pink colour. The mixture is divided into five or six Erlenmeyer flasks and incubated at 37° C. At the same time sterile agar plates are prepared and placed in the incubator with the flasks. After twelve, sixteen, or twenty-four hours the fluid in one or more of the flasks will be decolourised if the colon bacillus be present, and from the upper layer of the fluid a small loopful is removed and rubbed over one of the sterile agar plates, the process is repeated for the other colourless flasks, the plates incubated, and sub-cultures made from likely colonies.

¹ *Journ. Path. and Bact.* iv. 1897, p. 429.

² *Centr. f. Bakt.* xix. 1896, p. 13.

THE SPIRILLUM OF CHOLERA.

The Koch spirillum has been isolated by many observers from polluted water in infected districts. The method employed is to convert the water into peptone water by the addition of peptone and salt. Several Erlenmeyer flasks each receive 100 c.c. of the water, and to each is added 1 gram of peptone and 1 gram of salt. The flasks, which should be of such a size that the water forms a shallow layer not exceeding half an inch in depth, are loosely plugged with cotton-wool and incubated at 37° C. At intervals of ten, fifteen, and twenty hours a microscopical examination of the films is made to detect the presence of commas, and agar plates are prepared. If commas are found in numbers fresh peptone water may be inoculated, and they might also be tested for Widal's reaction with cholera serum. The plates and peptone-water cultures are further examined as detailed in Chapter XI.

5. *The Virulence of a Peptone-water Culture.*—If sufficient peptone and salt be added to a measured volume of the water to form a 1 per cent. solution of the former and a $\frac{1}{2}$ per cent. solution of the latter, the mixture incubated at 37° C. for twenty-four hours and injected intraperitoneally into a guinea-pig, a good water is stated not to kill, whereas a bad one does. The amount to be injected is 2 c.c., and death should ensue within forty-eight hours.

To recapitulate, the routine bacteriological examination of water comprises:—

1. The enumeration of the number of organisms present by means of gelatin plates, six or eight plates being prepared, and the ratio of liquefying to non-liquefying species.
2. The enumeration of the number of organisms which will develop at blood-heat by means of a couple of agar plates.
3. The search for the colon bacillus.
4. The pathogenic action of peptone-water cultures.
5. Special search for typhoid or comma bacilli.¹

¹ For further information on the bacteriological examination of water

Klein has laid some stress on the presence of anaërobic sporing bacilli and of the *balantidium coli* as evidence of sewage pollution, but their detection is of doubtful value.

The infection in enteric fever, cholera, and probably dysentery and malaria, is far more frequently water borne than conveyed in any other way. It might be supposed that the acid gastric juice would prevent this, and so perhaps it does in a large number of instances; but some experiments by Macfadyen¹ showed that, whereas in fasting animals, to which suspensions in water of the cholera spirillum were administered, living spirilla were present in the intestine, when the vehicle was milk none could be detected, the inference being that the gastric juice was secreted only when food was present, the water passing on to the intestine.

Reference has already been made to the removal of organisms in water by sand filtration. With regard to filters for domestic use, few of those in the market are capable of doing more than removing particles of suspended matter, while they allow from 5 to 50 per cent., or even more, of the bacteria present in the water to be filtered to pass through. Such filters are, of course, useless for the prevention of disease; in fact, rather favour it, by engendering a false sense of security; and when in use for some time without cleaning, the water after filtration may be worse, bacteriologically and chemically, than before filtration.

Woodhead and Wood² found that the only filters which were capable of completely removing organisms were the Pasteur-Chamberland, Berkefeld, and Porcelaine d'Amiante. The Berkefeld, while more rapid in action than the other two, after being in use for a few days may allow some organisms to appear in the filtrate. This, however, seems to be due

see *Micro-organisms in Water*, Percy Frankland, and *Reports of the Massachusetts Board of Health*.

¹ *Journ. of Anat. and Physiol.* xxi.

² 'Report on the Relative Efficiency of Water Filters,' *Brit. Med. Journ.* 1894, ii. p. 1053 *et seq.*

rather to a growth of organisms through the pores of the filter candle than to a direct passage. Lunt¹ found that while the ordinary water bacteria, such as the *bacillus fluorescens liquefaciens*, appeared in the filtrate from a Berkefeld filter within a few days of the infection of the sample, the typhoid bacillus and the comma bacillus similarly introduced had not passed through the filter four or five weeks after infection.

PROTOZOA AND ALGÆ IN WATER.

The examination of water for the minute forms of life other than the bacteria, and their enumeration, can be carried out by the Sedgwick-Rafter method.² A 6-inch glass funnel is plugged at the bottom of the stem with a perforated rubber cork, over the upper end of which a disc of fine silk bolting cloth, cut by a wad-cutter, is laid. Sharp, clean, dry quartz sand is then poured into the stem of the funnel to the depth of half an inch above the plug. The sand should be of such a size that the grains will pass through a sieve of 60 meshes to the inch, but not through one of 120 meshes. The sand is washed into place and thoroughly moistened with a little distilled water free from organisms.

The water to be examined is thoroughly shaken and 500 c.c. withdrawn and poured into the funnel; it runs through the sand, which detains any organisms it may contain. After the water has all passed through, the rubber plug is carefully removed and the sand washed down into a test-tube with 5 c.c. of distilled water. The contents of the test-tube are agitated and the tube is allowed to rest until the sand has deposited. Immediately this is the case the supernatant fluid is decanted into a second test-tube, carrying with it the organisms. One cubic centimetre of this is withdrawn by a pipette from midway between the top and bottom of this water and transferred to the counting plate. This consists of an ordinary glass slide on which a rectangular brass border (20 × 50 m.m.) is cemented, so enclosing exactly

¹ *Trans. Brit. Inst. of Prev. Med.* i. 1897.

² Calkin, *Twenty-third Annual Rep. of the State Board of Health, Massachusetts*, 1891. Pub. 1892.

1000 square mm. The brass border is 1 mm. thick, so that the cell contains exactly 1 c.c. It is covered with a cover-glass and examined with a low power. (For the exact details for the quantitative estimation the paper referred to must be consulted.)

THE BACTERIOLOGY OF AIR.

Just as in water, the bacteria in the air vary considerably at different times and seasons, under different conditions, and in various localities. The species met with are mostly saprophytes, consisting largely of chromogenic forms. A number of moulds occur (as spores), and, in fact, ordinarily are in large excess, together with yeasts and torulæ.

It is not easy for micro-organisms to become diffused through the atmosphere; they are incapable of a voluntary rising, and cannot be torn from a fluid or moist solid medium by a strong current of air. The medium on which they are growing must dry up completely and crumble into fine dust before they can be distributed through the agency of air currents.

The number of organisms in the air varies with the season, with rain, with altitude, with movement, &c. At Montsouris, Miquel found in one cubic metre of air 49 organisms in winter, 85 in spring, 105 in summer, and 142 in autumn. After heavy rain the air is much freer from organisms. Frankland found at Norwich Cathedral at an altitude of 300 feet seven organisms in two gallons, while on the ground eighteen were found; at the Golden Gallery at St. Paul's two gallons of air contained eleven organisms; in St. Paul's churchyard the number was seventy. On high mountains organisms are nearly absent from the air, and the same is the case at sea at a distance from land exceeding about 100 miles. Organisms are much fewer in the air of the country than in that of towns. At the entrance-hall, Natural History Museum, South Kensington, Frankland found in the

morning thirty organisms; in the afternoon, when many visitors were present, the number had risen to 293, showing the influence of movement. By keeping a volume of air absolutely still, enclosed in a box the walls of which were smeared with glycerin, Tyndall was able to completely free it from particles and organisms. The writer found from 43 to 150 organisms per ten litres of air in some of the principal streets of London.

BACTERIOLOGICAL EXAMINATION OF AIR.

A number of methods have been devised for the estimation of the number of micro-organisms in the air, of which the following are the chief:—

1. *Plate Method*.—Melted sterile nutrient gelatin is poured into a sterilised Petri dish, and allowed to set so as to form a sterile plate. This is then exposed to the air, by removing the lid, for a given time—one, five, ten, or fifteen minutes, &c.—the lid replaced, and the plate incubated at 22° C. for some days. The number of colonies of moulds, bacteria, yeasts, &c. is counted, and, having estimated the area of the gelatin plate,¹ the result is expressed as the number of organisms falling per square foot per minute. The results obtained by this method are roughly comparative, but no estimate can be formed from it of the number of organisms contained in a given volume of the air.

2. *Hesse's Method*.—This is a quantitative method for estimating the number of organisms contained in a given volume of air. The apparatus consists of a glass tube 30 inches long by $1\frac{1}{2}$ to 2 inches in diameter. One end of this tube is plugged with a rubber cork through which a glass tube passes, the other end is covered with a piece of sheet rubber perforated with a hole $\frac{1}{4}$ to $\frac{1}{2}$ inch in diameter;

¹ The area of a circular dish is calculated by multiplying the square of the diameter by 0.785.

over this is placed another sheet of rubber, unperforated. The small tube being plugged with cotton-wool, the whole is sterilised for an hour in the steam steriliser. Just before use 40 to 50 c.c. of melted sterile nutrient gelatin are poured into the tube, and its walls coated with the medium. The tube is then strapped horizontally on to a tripod stand, and the small tube connected by means of a piece of rubber tubing to an aspirator consisting of two flasks arranged so as to form a reversible syphon. A litre of water is poured into the flask connected with the tube, and having removed the outer sheet of rubber from the end of the tube, the water is syphoned over to the second flask, placed at a lower level, and in doing so draws through the tube an equal volume of air. The second flask is then connected with the tube, and the position of the flasks being reversed the water is again syphoned over and a second litre of air passes through the tube, and this process is repeated until 5, 10, 15 or 20 litres of air have been drawn through the tube. The rate of flow is controlled by a screw-clamp on the rubber connecting tube; it should not exceed half a litre per minute. With this rate of flow all the organisms are deposited on the gelatin coated tube. The aspiration being completed the rubber tube is disconnected and the sheet of rubber replaced over the end of the tube, which is then incubated, and the colonies counted when they have developed.

3. *Petri's Method*.—Petri aspirates the air through a glass tube containing sterilised sand, kept in place by fine wire-gauze wads. When the sample has been taken, the sand is distributed in Petri dishes, and melted sterile gelatin is poured over it and allowed to solidify, plate cultures being thus prepared. The objection to this method is the presence of the opaque particles of sand in the culture medium.

4. *Frankland's Method*.—The air to be examined is aspirated through a tube 5 inches in length and $\frac{1}{4}$ inch in diameter (fig. 74). One end of the tube is open, the other

(c) is plugged with cotton-wool. At a distance of 1 inch from the open end the tube is slightly constricted to support a plug of glass-wool (A). At a distance of $2\frac{1}{2}$ inches from this plug the tube is again constricted to support a second plug (B), consisting of glass-wool and finely powdered cane-sugar, supported in front and behind by plugs of glass-wool. Several of such tubes having been prepared, they are placed in a tin box and sterilised at 130°C . for three hours, and can then be easily transported without risk of contamination. When required for use, a tube is quickly removed from the box, being handled by the plugged end, which is connected by stout rubber tubing to aspirating flasks such as is used in Hesse's apparatus. The tube is clamped horizontally to a retort stand, and by attach-



FIG. 74.—FRANKLAND'S TUBE FOR AIR ANALYSIS.

ing the second flask to a small hand exhaust pump, the water can be syphoned over from the first flask, a corresponding volume of air passing through the tube. When the desired volume of air has been aspirated through the tube, it is disconnected and placed in another sterile tin box. As many tubes as desired can be employed to control each other or to examine the air in different localities and under different conditions. All the samples having been taken, the tubes are manipulated on returning to the laboratory. The tubes, as before, being handled by the ends only, a file mark is made across the centre of each tube, which is then broken in half and the plugs of glass-wool and sugar shaken, or pushed by means of a sterile wire, into a sterile flask of about 250 c.c. capacity. Into this is then introduced 10 or 15 c.c. of liquefied sterile nutrient gelatin; the sugar dissolves, the glass-

wool becomes disintegrated, and a roll-culture is made on the walls of the flask, which is incubated at 22°C ., and the colonies counted when they have developed.

Sedgwick and Tucker's Method.—One of the best and most convenient methods for the bacteriological examination of air. A glass tube of special form is employed (fig. 75); this consists of an expanded portion (A) about 15 cm. long and 4.5 cm. in diameter; one end of this is contracted so as to form a neck 2.5 cm. in diameter and in length; to the other end is fused a glass tube (B C) 15 cm. long and 0.5 cm. in diameter. The neck of the tube is plugged with cotton-wool, and two wool plugs are inserted in the narrow tube, one at its open end, the other (C) about 6 to 8 cm. from the wide part. The whole is then sterilised. When cool, the narrow part of the tube, from its origin at the wide part down to the first plug (B C), is filled with powdered cane-sugar (No. 50, B.P. gauge) which has been carefully dried and sterilised at 120° to 130°C . The tube is again sterilised at 120° to 130° for two or three hours, the greatest care being taken not to melt the sugar. After sterilisation the tube is ready for use. The wool plug is removed from the mouth and a measured volume of air is aspirated through the layer of powdered sugar by means of a small hand air-pump, the volume of air being measured by the displacement of water in a flask. Having taken the sample (5 to 20 litres), the wool plug is replaced in the neck. The powdered sugar is then shaken down into the wide part of the tube (A), and 15 c.c. of melted sterile nutrient gelatin are poured in. The powdered sugar readily dissolves



FIG. 75.—SEDGWICK AND TUCKER'S TUBE FOR AIR ANALYSIS.

in the melted gelatin, and when solution is complete a roll-culture is made in the tube, just as in Esmarch's method. The tube is then placed in an incubator at 20° C., and the colonies are allowed to develop.

In both Frankland's and Sedgwick and Tucker's methods the sugar, after powdering and sifting and before introducing into the tubes, should be thoroughly dried by keeping in the warm incubator for several days with occasional stirring. Unless this be done, the sugar is apt to cake and discolour during sterilisation.

SOIL.

The upper layers of soil contain large numbers of organisms, chiefly bacilli. The species are very varied; among pathogenic ones may be named the bacilli of tetanus and of malignant œdema. The *bacillus mycoides* is very abundant, and the varieties of proteus, the hay, and potato bacilli, are common, while the nitrifying are of course present.

Below five or six feet aerobic organisms are scanty, but the anaërobic and thermophilic ones are still met with. The number of organisms present in soil is variable from 200,000 to 45,000,000 in ordinary earth, while in dirty and busy streets there may be as many as 1,000,000,000 per gram.

The bacteria in the soil may be examined by adding traces of the soil to sterile nutrient broth, thoroughly crushing and soaking it, and then making plate or roll cultures, aerobic and anaërobic.

To make anything like an accurate quantitative examination is almost impossible. Weighed amounts of the soil, after thorough pulverisation in an agate mortar, may be introduced into sterile test-tubes and thoroughly exhausted by repeated washing with sterile water or broth, plate cultivations being made with the washings.

Various boring apparatus have been devised for withdrawing soil from various depths.

SEWAGE.

Sewage is exceptionally rich in organisms, but the numbers present are variable. Jordan in Massachusetts found an average of 708,000 per cubic centimetre. Laws and Andrews¹ found from 905,000 to 11,216,000, the latter being the highest number obtained. The number of organisms naturally varies at different seasons and with the amount of dilution. The organisms present are very varied; moulds, yeasts, and sarcinæ are of occasional occurrence. A few micrococci are met with and a streptococcus (*streptococcus mirabilis*, Roscoe and Lunt), but bacilli, especially liquefying forms, largely predominate. Many anaërobic sporing bacilli are also found. The commonest species are the *bacillus fluorescens liquefaciens* and varieties, several varieties of proteus, the *bacillus filamentosus*, the varieties of *bacillus mesentericus*, *bacillus mycoides*, *bacillus subtilis*, and the colon bacillus. The latter numbers from 20,000 to 200,000 per c.c., and the other bacilli mentioned number 200,000 to 2,500,000 per c.c. Bacteria introduced into sewage are probably soon suppressed by the predominant species in the sewage.

The air of well-ventilated sewers differs but little from that of the external air, and the organisms in it contrast with those of sewage by the abundance of moulds.

The powerful liquefying and solvent actions of the bacteria in sewage have suggested a means of dealing with sewage so as to make use of these properties, and render sewage disposal an automatic process. Such are the Scott-Moncrieff and septic-tank processes. In the former the sewage is led by channels through beds of flint and coke, by which a large surface is presented to the organisms; in the latter the sewage slowly passes through a series of tanks, and gradually becomes dissolved and purified. In each case the beds or tanks have to be given a rest every twenty-four hours or thereabouts to allow of recovery, so that two or three sets are required, some resting while the others are working.

¹ Reports to the London County Council on: I. The Micro-organisms of Sewer Air; II. The Micro-organisms of Sewage. 1894.

EXAMINATION OF SEWAGE.

To ensure a fair average sample the sewage should be collected in small portions at intervals. The portions are mixed, strained through muslin, and after dilution examined by means of plate cultures. Two dilutions should be made—one a thousand, the other ten thousand times—and sterile water, or preferably the fluid obtained by filtering sewage through a Berkefeld filter, used as the diluting medium. Several sets of gelatin and agar plates should be prepared, with measured volumes of the dilutions, and to enumerate all the organisms some anaërobic plates must also be prepared. When they have developed the colonies are counted and examined.

MILK.

Milk is an admirable nutrient soil for the development and multiplication of micro-organisms, and as delivered to the consumer may contain an appalling number of bacteria. In ordinary milk there are from 1,000,000 to 5,000,000 bacteria per c.c., and it frequently contains 10,000,000 to 15,000,000, with an average of about three to four millions. Cream is even richer in bacteria than milk, and averages about 8,000,000, and may contain as many as 30,000,000 organisms per c.c.¹ Although all the ordinary species may be met with, milk has largely a bacterial flora of its own, comprising many forms producing lactic and butyric acid fermentations. Organisms also occur having more or less specific effects, and giving rise to bitter milk, viscid milk, &c. The lactic ferments are largely non-sporing, the butyric acid ones chiefly sporing species. The commonest of the lactic ferments is the *B. acidi lactici*, which is non-liquefying, a little larger than the colon bacillus, and producing growths something like those of the latter organism, but the film on gelatin is much denser. Another common lactic organism

¹ Russell, *Centr. f. Bakt.* 2te Abt. i. 1895, p. 741.

is the *oidium lactis*, a mycelial form, the growth of which forms little fluffy tufts. In addition to the organisms named, pathogenic species may also be met with, viz., the tubercle, diphtheria, typhoid, and comma bacilli, and the *S. pyogenes aureus* and *streptococcus pyogenes*. Scarletina and the foot and mouth disease can also be conveyed by milk, and the diarrhoea of infants is largely due to the use of unsterilised milk. In order to render milk wholesome for infants and free from infective organisms two methods are adopted, sterilisation and pasteurisation. To ensure sterilisation it is necessary to heat the milk to boiling point for six hours, or for a shorter period to steam under pressure. Such treatment, however, markedly alters the flavour of the milk, and is said to diminish its nutritive value. If the milk be heated to a temperature not exceeding 70° C. the flavour and nutritive qualities are unaltered, while the pathogenic species are all destroyed. This method is termed 'pasteurisation,' and consists in heating the milk to 68° C. for 20 to 30 minutes.¹ Pasteurisation destroys 99·75 per cent. of the total number of organisms.²

EXAMINATION OF MILK.

The bacteriological examination of milk is conducted by means of gelatin plate cultures. The milk is diluted 1000, 10,000, or even more times with sterile water, several sets of plate cultures made, and the colonies counted as late as possible, to allow the slowly growing ones to develop.

The detection of pathogenic organisms, with the exception of the tubercle bacillus, is a difficult and very uncertain matter.

1. The detection of the tubercle bacillus has been described at page 211. A more elaborate method is to mix 20 c.c. of the milk with 1 c.c. of a 50 per cent. potash solution, and heat in a water-bath until the solution turns brownish; 20 c.c. of acetic acid are added. The mixture is shaken, heated in a water-bath

¹ 'On the Pasteurisation of Milk.' See a paper by Macfadyen and the writer, *Trans. Brit. Inst. Prev. Med.* i. 1897, p. 82. (Refs.)

² Russell, *Centr. f. Bakt.* 2te Abt. i. 1895, p. 741.

for three minutes, and centrifugalised for ten minutes. The fluid is poured off, 30 c.c. of hot water added to the sediment, and again centrifugalised. The cover-glasses are then prepared from the sediment, and stained for the tubercle bacillus.¹

2. The diphtheria bacillus can be detected with certainty only by the inoculation method (p. 186).

In milk and cheese a bacillus is frequently met with closely resembling the diphtheria bacillus in its morphological and cultural characters ; it is, however, quite non-pathogenic.²

3. The typhoid and comma bacillus may be examined for by the methods given for water (pp. 382 and 386).

4. The *S. pyogenes aureus* and the *streptococcus pyogenes* may be examined for by means of cultures and plate cultures on glycerin agar.

Meat is not likely to convey any infective disease with the exception of tuberculosis and anthrax. It may be examined by cultures and plate cultivations, and by inoculation and feeding experiments.

Bread.—Troitzki states that new bread contains no micro-organisms, but Waldo and Walsh found that such organisms as the comma bacillus were not destroyed by passing through the ordeal of the baker's oven. Cut bread forms a good nidus for the development of pathogenic organisms.

Butter contains from two to forty-seven millions of micro-organisms per gram. Tubercle bacilli have been found in butter, and the comma bacillus artificially introduced survives for over a month.

SOME OF THE COMMONER ORGANISMS OF WATER, AIR, AND SOIL.

Micrococcus agilis.—Occurs principally as a diplococcus; forms also short chains, and is found sometimes in tetrads. Motile, possessing very long and fine flagella. On gelatin it produces a pinkish-red growth and liquefies very slowly. On agar and potato it also produces a pinkish-red pigment. It does not grow at 37° C. Found chiefly in water.

¹ See *Nature*, 47, 1893, p. 254.

² See *Scientific Bull.* No. 2, Health Dept., City of New York, 1895, p. 10.

Micrococcus candicans.—A coccus of irregular size, occurring in groups. It is non-motile, and forms no characteristic arrangement. Liquefies gelatin with a white opaque deposit. On agar it forms a dazzling white mass. Broth becomes turbid and a white deposit forms. Non-pathogenic.

Sarcina lutea.—Very large cocci arranged in twos and fours. It stains by Gram's method, does not form spores, and is non-motile. A marked cubical arrangement of the cells is well seen in a hanging-drop specimen. A yellow growth forms on gelatin, which is slowly liquefied. On agar it forms a thick chrome-yellow, shining streak. On potato the colonies are of a sulphur-yellow colour and grow very slowly. In broth a yellow deposit forms in the depth, without film formation. Common in air and water.

Pink Torula.—An organism frequently occurring in air and water. It will be easily recognised by the forms of the cells, which are yeast-like and show budding. Produces a pink growth on agar, potato, and gelatin, without liquefaction of the latter. A white and a black torula, and many yeasts, are also frequently met with in air and water.

Bacillus filamentosus.—A longish rod, forming filaments of ten to twenty or more individuals. Non-motile; forms spores in the centre of the rod, markedly aërobic. Liquefies gelatin rapidly, the liquid gelatin remaining clear and a whitish sediment falling to the bottom. On agar it grows well at 20° to 22° C., but not at 37° C.; it produces a very wavy, feathery growth. Does not stain by Gram's method. Non-pathogenic. Isolated by Klein from sewage.

Bacillus fluorescens liquefaciens (Flügge).—A short rod, occurring singly and in pairs. It is very motile, forms no spores, and does not stain by Gram's method. It yields an acid reaction in milk and completely peptonises it. It liquefies gelatin very rapidly; the liquefied gelatin is of a whitish colour at first, finally becoming a fluorescent green. On agar it forms a greenish-yellow fluorescent layer. On potato it forms a brownish growth. It is not pathogenic. One of the commonest organisms in water, and of which there are many varieties, some not liquefying gelatin, *B. fluorescens non-liquefaciens*.

Bacillus megaterium.¹—First described by De Bary. A large rod, forming filaments. Motile, possessing several long flagella. Endospores freely formed, of same diameter as rods. Stains by Gram's method. On agar it forms a thick cream-coloured growth, at first much like that of anthrax. Gelatin is rapidly liquefied, with a yellowish flocculent deposit. General turbidity in broth; grows at 20° to 37° C. Not pathogenic.

Bacillus mesentericus vulgatus ('Potato bacillus').—A small plump rod with rounded ends, often forming long filaments. It is very motile, forms large ovoid spores, and does not stain by Gram's method. Polar staining may occur. Gelatin is rapidly liquefied. Produces a dirty white, dry, crinkled growth on agar. On potato it grows rapidly, producing a very wrinkled, dry film, slightly pinkish in colour. Broth is rendered turbid and a film grows on the surface.

There are other bacilli having the general characters of the *B. mesentericus vulgatus*, but differing in the colour of their growths; they are the *B. mesentericus fuscus*, *ruber* and *niger*.

Bacillus mycoides.—A rod of varying length, forming large spores. It is motile, and does not stain by Gram's method. Forms a white, cloudy patch on gelatin, liquefying it in time. Produces a tufted, mould-like growth on agar, and on potato a slimy growth. Very abundant in the soil.

(*Bacillus*) *Proteus vulgaris*.—A bacillus of varying length, and occurring in spirillar-like threads and marked involution forms. Very motile, possessing long flagella. Does not form spores and does not stain by Gram's method. On agar it forms a greyish-white film, slimy and thin. Liquefies gelatin very rapidly, and a thick sediment deposits at the bottom of the liquid. In gelatin plates the bacilli form parallel groupings in the colonies, which are of a yellowish-brown colour, curiously irregular and convoluted, and move slowly over the medium.

¹ The specific name is variously spelt 'megaterium' and 'megatherium.' The former occurs in all the earlier papers and in De Bary's works, the latter in some recent ones, notably in Flügge's *Die Microorganismen*. 'Megatherium' is evidently derived from 'megatherion,' which is used by Aristotle to denote a little worm or insect. The derivation of 'megaterium' is not so obvious, but Dr. Macfadyen has suggested that it is a contracted form of 'megabakterion,' a large rod, on account of its size. This seems very probable, and I have preferred to retain the old spelling.

It is pathogenic to rabbits and guinea-pigs, giving rise to abscesses when injected subcutaneously.

There are other species of proteus, viz.—*mirabilis*, and *Zenkeri*, differing in the rate of liquefaction of gelatin. The *bacterium termo*, *bacterium Zopfii*, and *B. saprogenes* of Rosenbach are, probably, 'proteus' species. They occur in air, water, and sewage, and are active agents in the putrefaction of proteids (Flügge).

Bacillus prodigiosus.—A short rod, often almost coccoid, frequently linked in pairs. Feebly motile, does not form spores, and does not stain by Gram's method. Liquefies gelatin very rapidly, forming an abundant flocculent deposit of a deep crimson colour. On agar it also grows very rapidly, producing a very deep red colour if incubated at 20° to 22° C. ; but if grown at 37° C. the colour is not nearly so intense. It produces a growth on potato of a brilliant red colour, which has a metallic lustre upon it. Broth is rendered turbid without film formation. Found chiefly on moist bread, potatoes, and in water and air. The pigment is insoluble in water, but is soluble in absolute alcohol, ether, chloroform, and benzol. Injected into the peritoneal cavity of guinea-pigs in large amount the organism produces death.

Bacillus subtilis (hay bacillus).—A rod much resembling anthrax, the difference being that it has rounded ends, while anthrax possesses square ones ; grows into long filaments. It is motile, possesses several flagella, and forms spores. It is very resistant, and the spores will withstand a temperature of 120° C. for an hour without being killed. Liquefies gelatin rapidly, forming a pellicle on the surface. Broth becomes turbid with a pellicle on the surface. Liquefies blood serum rapidly. On agar forms a dryish, crinkled film. Abundant in hay, straw, dust and earth. Non-pathogenic.

Bacillus violaceus.—A rod variable in size. Very slightly motile, and does not form spores. On gelatin the growth is at first white, later on a violet blue ; liquefies slowly. Grows fairly well on agar, producing, at 22° C., a violet, moist, thick layer. On potato it forms a rich violet colour. Grows much better and produces a better colour at 20° to 22° C. than at 37° C.

CHAPTER XXII.

ANTISEPTICS AND DISINFECTANTS.

A LARGE number of substances variously known as germicides, antiseptics, disinfectants, deodorants, &c. have the power of interfering with, or masking the results of, the vital activities of micro-organisms. Germicides are substances which kill bacteria, or germs; antiseptics, by inhibiting bacterial development, prevent sepsis, or putrefaction; and by disinfectant is meant a substance which prevents the action of, or destroys, infective matters, while deodorants destroy or absorb foul-smelling gases, the result of putrefaction and similar processes. All germicides are disinfectant and antiseptic, but many antiseptics, though preventing or inhibiting the development of bacteria, are not necessarily germicidal.

In addition to chemical or other substances for preventing bacterial development, other methods can also be employed for destroying or excluding micro-organisms. Such are heat, dry and moist, light, desiccation, and filtration.

Heat.—Burning is, of course, by far the most efficient form of destroying infective matters, and should always be employed where possible, as for rags, old clothing, or bedding, and for the carcasses of animals.

Dry heat may also be used, and forms the basis of some disinfectors (Ransome's), but is not nearly such an efficient means as moist heat. The objections to dry heat are, that to insure the destruction of bacteria and spores

the temperature must be high and the heating prolonged. Koch and Wolfhügel found that two hours at 150° C. did not always ensure sterilisation, and Gaffky and Löffler state that the spores of some organisms are killed only by exposure to hot air at 140° C. for three hours. Moreover, dry heat has little power of penetration, and it requires many hours for the centre of a mass of bedding, or the like, to attain a temperature requisite for sterilisation; while some articles and fabrics are distinctly injured by the prolonged heating. The highest temperature which can be safely adopted for a dry-heat disinfector is about 120° C., and even then if large masses have to be treated the heating has to be continued for from eight to ten hours. A rise of 5° C. above this temperature is sufficient to damage many woollen goods, which enhances the objections to a dry-heat disinfector, as it is difficult to keep the temperature of a large chamber constant.

For the reasons given above, disinfection by dry heat is often impracticable; on the other hand, *moist heat* is more effective, is found to work well in practice, and is now generally adopted. In the household, for articles which cannot be burnt, brisk boiling for an hour or so will be efficient.

For public disinfectors steam under pressure is employed, as in the Washington-Lyons apparatus. Steam under pressure has not such a deleterious action on articles, with the exception of leather, as dry heat, while its penetrating powers are far greater. The Washington-Lyons apparatus, or its modifications, is an elongated cylindrical or rectangular boiler with double walls, forming a jacket, and a door at each end. The chamber is of sufficient size to admit bedding, and is built into the partition wall between two rooms, so that each door opens into a different room. Into one of the rooms the infected articles are conveyed, and are placed in the disinfector as lightly packed as possible; when disinfected they are removed by the opposite door into the other room, thereby

avoiding all chance of reinfection ; steam at a pressure of about 20 lbs. is admitted into the jacket and then passes to the inner chamber, the object of the jacket being to warm the chamber, and so prevent condensation. For the same purpose hot air is sometimes injected beforehand to warm the chamber and articles, and after the steam disinfection, can again be injected for drying. The length of time required for disinfection does not exceed a half to one hour.

Light.—Light is not used directly for disinfection, but indirectly in nature and in our homes may not be an inconspicuous factor. It has previously been referred to at p. 15.

Desiccation, although one of nature's methods of disinfection, is not made use of to any extent by man, but for the prevention of sepsis in, and preservation of, many articles of food is largely employed.

Filtration is a method of disinfection by exclusion, and in the form of sand filtration and filtration through porous porcelain, as in the Berkefeld and Pasteur-Chamberland filters, is made use of for the sterilisation of water and other fluids.

The deodorants act largely mechanically, and although often not germicidal, and hence not ideal disinfectants, are of considerable value in preventing the deleterious and depressing effects of the emanations from decomposing organic matter. Such are charcoal, ashes, dry mould, and peat. Other deodorants, such as quicklime and chloride of lime, act chemically.

Germicides and Antiseptics.—The germicides and antiseptics may be considered together, for although many antiseptics are not germicidal, all the germicides in small amounts act as antiseptics. The principal germicides and antiseptics are the halogen elements, chlorine, bromine, and iodine, the mineral acids, a large number of metallic salts, many coal-tar derivatives, and various organic bodies and essential oils.

Chlorine acts to a large extent by oxidation. It is not

often used in solution, but in the gaseous form has been much employed for fumigating rooms. At least 0·5 per cent. must be present, but even then it acts slowly.

Chloride of Lime is one of the best substances for household disinfection. It may be sprinkled over decomposing matters or used in solution, 1 per cent., which is more active at a temperature 40° to 50° C. It has been recommended by Delépine for the disinfection of houses which have been inhabited by tuberculous patients.

Oxides of Chlorine are generated in the electrolysis of sea-water, in the Hérmite process, for the treatment of sewage, but Roscoe and Lunt criticised the method unfavourably. Oxides of chlorine are also generated by treating potassium chlorate with hydrochloric acid, a mixture which has been used internally in the treatment of infective diseases.

Hydrochloric Acid is a valuable disinfectant both in solution and in the gaseous state.

Bromine is not such a powerful disinfectant as chlorine; it may be used in solution of 1 in 1000—1 in 2000.

Iodine is less active than bromine, and is costly.

Iodine Trichloride is a very powerful disinfectant, and may be used in solution of 1 in 1000.

Chloroform is a powerful antiseptic, but at least 1 per cent. must be present to act as a germicide; it is costly, and not much used as a practical disinfectant, but in bacteriological and physiological chemistry is a useful antiseptic for preserving solutions which putrefy easily.

Iodoform is valuable for dusting wounds, though its penetrating odour is objectionable, and has led to the introduction of many substitutes. Its value as an antiseptic has been greatly discussed; micro-organisms will develop in nutrient media containing a considerable proportion, but probably when in contact with bacterial toxins a decomposition is effected, hence its value.

Sulphuric Acid, when concentrated, destroys any micro-organism it comes in contact with, and as little as 0·05 per cent. is sufficient to destroy the comma bacillus of cholera.

Sulphurous Acid is the commonest disinfectant employed for fumigation; it is generated by burning sulphur or sulphur candles, or from the liquid. At least 1 per cent. must be present, and the air must be moistened. Kenwood found that the diphtheria bacillus was inhibited by 0·25 per cent. of SO_2 , and killed when the amount of gas exceeded 0·5 per cent.¹

Nitric Acid is an active disinfectant, and when mixed with sulphuric acid yields oxides of nitrogen, which can be used for fumigation.

Boric Acid is a feeble germicide, but is useful as an antiseptic. Being comparatively tasteless, and having little action on the substances with which it is mixed, it has been largely used as a preservative for articles of food.

Ozone when moist is a powerful germicide, but probably at least 5 per cent. is required to be efficient; it has been proposed to employ it to sterilise water, but no practical means have yet been devised to accomplish this economically.

Peroxide of Hydrogen is antiseptic only when present to the extent of 0·1 per cent. It is mostly used as an application to foul wounds and ulcers, and not as a general disinfectant.

Zinc Chloride is a powerful antiseptic, a $2\frac{1}{2}$ per cent. solution being germicidal. Burnett's disinfecting fluid contains about 82 per cent. A 5 to 8 per cent. solution has been used for treating wounds.

Cupric Sulphate is germicidal in quantities of 1 per cent., and antiseptic in 1–1000. It has been used for disinfecting cesspools, bed-pans, &c. in cholera and typhoid fever.

Ferrous Sulphate is moderately antiseptic, 1 per cent. being required to prevent putrefaction. It has been used

¹ *Brit. Med. Journ.* 1896, i. p. 439.

for the same purposes as cupric sulphate, and has been recommended by the Local Government Board, in strong solution or in powder, for disinfecting the contents of privies and ashpits.

Potassium Permanganate as a germicide is only applicable in strong solution (5 per cent.), for its disinfecting power is rapidly exhausted by the reducing action of organic matter. The well-known Condy's fluid is a solution of this substance.

Mercuric Chloride (corrosive sublimate) is one of the most powerful germicides and antiseptics. As little as 1-10,000 is antiseptic, and 1-500 kills bacterial spores. Many conditions, however, modify its power, and in practice these amounts may not be found efficient; for example, it is precipitated by albumin, and is readily converted into an insoluble sulphide by the sulphur of organic bodies, and thereby rendered inert. Dilute solutions, unless prepared with distilled water and kept from access of air and light, are unstable. The addition of $\frac{1}{2}$ per cent. hydrochloric acid to the solution renders dilute solutions stable, and to a large extent prevents precipitation by albumin. Tartaric acid and 10 per cent. sodium chloride act similarly as regards the precipitation of albumin.

The Local Government Board recommends the following solution for disinfecting purposes :—

Corrosive Sublimate	.	.	.	$\frac{1}{2}$ oz.
Hydrochloric Acid	.	.	.	1 oz. fl.
Anilin Blue	.	.	.	5 gr.
Water	.	.	.	3 gals.

This forms a solution of 1-1000 nearly; it would be preferable to use 1 oz. of corrosive sublimate.

Mercuric Iodide dissolved by means of an excess of potassium iodide forms a very powerful disinfecting solution, and is stated to be twice as potent as mercuric chloride, while

it is not so poisonous, and does not precipitate albumin. It has the disadvantage of corroding steel instruments.

Zinc Mercuric Cyanide was introduced by Lord Lister as an antiseptic dressing, which should contain 3 to 5 per cent. of the powder. It is insoluble in water, but a small amount, sufficient to be powerfully antiseptic, dissolves in the albuminous discharge from wounds. It is less irritating than mercuric chloride and mercuric iodide.

Silver Nitrate, Gold Chloride and Cyanide, and Osmic Acid are all powerful germicides, but their cost is prohibitive to their general use.

Carbolic Acid is perhaps one of the most generally used disinfectants; it is cheap, somewhat volatile, and penetrating, all of which are advantages. One part in 400 is antiseptic, and 1-20 germicidal, though to destroy spores may require long treatment.

Cresol, the next higher member of the phenol (carbolic acid) series, is also a good disinfectant, and forms the active principle of creosote and of a number of patent disinfecting fluids, viz. Lysol, Jeyes' Fluid, Creoline, and Izal, all of which seem to be efficient antiseptics.

Anilin Dyes.—Some of the anilin colours, especially the purified methyl violet or pyoctanin, have been claimed to be powerfully antiseptic in solutions 1-500 to 1-1000.

Salicylic Acid has considerable antiseptic but little germicidal power, and has been used as a preservative for articles of food. The same may be said of *Benzoic Acid*.

The Essential Oils, *Peppermint, Mustard, and Cloves, Thymol* and *Menthol* are powerfully antiseptic.

Sanitas prepared by the oxidation of turpentine is about equal in antiseptic power to the latter, and, being non-poisonous, miscible with water, and non-corrosive, it is useful in many cases:

Alcohol is not a very reliable antiseptic, about 10 per cent. being required to inhibit putrefaction. As a germicide

it is not very powerful, absolute alcohol destroying the *S. pyogenes aureus* only after an exposure of an hour or more.

Formalin is a 40 per cent. solution of formaldehyde in water. It is one of the most powerful antiseptics and germicides we have. According to Slater and Rideal, from 1 in 5000 to 1 in 20,000 inhibits the growth of bacteria, and a 1 per cent. solution destroys non-sporing bacteria in thirty to sixty minutes. It is volatile and can be used for fumigating purposes, and is perhaps the only germicidal vapour that can be applied for practical disinfection. Its cost, however, is somewhat prohibitive. Both the vapour and solution are very irritating.

A number of conditions modify germicidal and antiseptic actions; cold retards, while warming intensifies them, and substances present which precipitate the active constituent in an insoluble form may completely destroy the efficacy of a disinfecting solution.

Foreign substances present with the antiseptic may also diminish or neutralise its properties; such are oil, alcohol, and glycerin. For example, carbolic oil possesses little antiseptic power, and glycerin when present to the extent of 50 per cent. is inhibitory to carbolic acid and corrosive sublimate. Antiseptic ointments should be made with vaseline or, better still, with lanoline, and not with lard.

Paul and Krönig¹ have made a number of experiments on the *S. pyogenes aureus*, and spores of anthrax, with a view of determining the effects of various acids, bases, oxidising agents, and metallic salts on bacteria. The salts of mercury, gold and silver exert a marked germicidal action, strongest in the case of mercury, while the platinum salts are almost inactive. The effect of mercuric chloride is markedly lessened by the presence of sodium chloride, or other chlorides. Of the oxidising agents, nitric, chromic, chloric and permanganic

¹ *Zeitschr. f. physikal. Chem.* 1896, 21, p. 414.

acids act in the order stated; chlorine has the most powerful action of the halogens. Phenol acts better in a 5 per cent. solution than in higher concentrations, and the effect is increased by the addition of sodium chloride, but diminished by the presence of alcohol, and under the most favourable conditions it is not nearly such a powerful germicide as mercuric chloride. Mercuric chloride dissolved in absolute alcohol has little or no effect. Organisms in masses are less readily acted upon by antiseptics than when they are isolated.

TABLE SHOWING ANTISEPTIC AND GERMICIDAL POWER OF VARIOUS SUBSTANCES ACCORDING TO DIFFERENT OBSERVERS, WITH TIMES OF EXPOSURE.

—	Antiseptic Action	Germicidal Action on Non-sporing Forms	Germicidal Action on Anthrax Spores
Mercury, biniodide .	1 : 20,000	—	—
Mercury, perchloride	1 : 10,000	1 : 2000 to 1 : 5000 half an hour	1 : 500 to 1 : 1000 one hour
Gold, trichloride .	1 : 40,000	1 : 500 to 1 : 1000 two hours	—
Chloride of lime .	—	1 : 100 a few min.	5 : 100 half an hour
Carbolic acid . .	1 : 400 to 1 : 700	1 : 100 to 1 : 200 two hours	5 : 100 at 22° C. does not kill after several days' exposure
Sulphurous acid (gaseous)	—	1 : 100 to 4 : 100 (moist) four to twelve hours	Does not kill
Chlorine (gaseous) .	—	1 : 2500 to 1 : 200 one to three hours	1 : 100 in three hours
Zinc chloride . .	1 : 500	1 : 200 to 2 : 100 two hours	Does not kill
Iodine, trichloride .	—	—	0·2 : 100 few min.
Oil of Mustard } Oil of Peppermint }	1 : 30,000	—	—
Salicylic acid . .	1 : 1000	1 : 400 to 2·5 : 100 half to six hours	Does not kill
Quinine	1 : 900	Does not kill . .	Does not kill
Formalin (fluid) .	1 : 5000 to 1 : 20,000	1 : 100 one hour	15 : 1000 one and a half hours
Formalin (gaseous) .	—	1 : 20,000 (probably much too little)	—
Boric acid . . .	1 : 150	Saturated solution does not kill pus cocci in five hours	—
Potassium permanga- nate	1 : 300	1 : 100 two hours .	5 : 100 twenty- four hours

It is useless to add a small quantity of antiseptic to a large volume of fluid or solid; the antiseptic must be added in sufficient amount so that the mixture contains the minimum percentage which has been found by experiment to be efficient. For this reason the disinfecting of sewers, sewage, streets, &c. by relatively small quantities of antiseptics is useless, and the money so wasted would be far better employed in providing more water for flushing purposes.

In medical practice, while antiseptics can be applied locally with success and for disinfecting the alimentary tract, &c. no substance has yet been discovered which can be administered with safety to such an extent as to saturate the body and so exert a general germicidal action in infective diseases.

In surgical practice no unbiased observer can doubt the efficacy of antiseptic treatment, but many so-called 'antiseptic operations' are marred by faults of omission and commission which render them far from being so. Recently there has been some controversy between the advocates of 'antiseptic' and 'aseptic' surgery. Undoubtedly antiseptics do diminish the vitality, and therefore the reparative power, of the tissues, and aseptic methods may, to some extent, take the place of antiseptic ones, but for the final dressings it can hardly be doubted that the antiseptic are the safer. For it is impossible to exclude micro-organisms altogether from wounds, and if they be present they will be able to grow and multiply under an aseptic dressing, but would be prevented by an antiseptic one.

THE BACTERIOLOGICAL DETERMINATION OF ANTISEPTIC POWER.

1. For the determination of the value of an antiseptic, Koch originally used the thread method. Sterilised silk threads are impregnated with sporing and non sporing organisms, lightly

dried, and then exposed to the action of the antiseptic solution of a known strength for a given time. The threads are then thoroughly washed with distilled water to remove the antiseptic, and sown on the surface of agar or other suitable culture medium. If no growth occurred the organisms were assumed to have been destroyed. As a matter of fact, however, it is extremely difficult to get rid of the last traces of the antiseptic, which may inhibit growth although the organisms may yet be alive, a fallacy which caused an exaggerated value to be assigned to many substances—for example, corrosive sublimate. The thread method may still be employed, but after treatment the threads should be sown in bouillon, or, better still, if pathogenic organisms be the subject of experiment, inoculated into a susceptible animal.

2. The most satisfactory method is to employ a known volume of a recent bouillon culture of an organism, to which is added a measured amount of the antiseptic. After acting for a given time, loopfuls of the mixture are sown in sterile bouillon tubes. If a growth occurs the organisms have not been destroyed.

3. Volatile disinfectants may be tested by moistening the wool plug of an agar tube, inoculating the agar, and capping with a rubber cap, and observing whether any growth occurs.

4. Volatile disinfectants may be tested by exposing silk threads, impregnated with organisms, some free, others done up in packets of cotton-wool, in a room or chamber of known cubic capacity, to the action of the gas ; a known amount of which is present in the chamber. After exposure for a given time, the threads are sown on agar tubes, and the tubes incubated.

APPENDIX.



I.—WEIGHTS AND MEASURES.

1 cubic centimetre (1 c.c.)	= 16 minims nearly (16·23 m.).
1 fluid drachm	= $3\frac{1}{2}$ cubic centimetres nearly (3·55 c.c.).
1 fluid ounce	= 28 cubic centimetres nearly (28·397 c.c.).
1 litre	= 35 fluid ounces nearly (1·76 pints).
1 pint	= $\frac{1}{4}$ litre or 568 c.c.
1 gram (1 grm.)	= $15\frac{1}{2}$ grains nearly (15·432 gr.).
1 drachm (Apothecaries')	= 4 grams nearly.
1 centimetre	= 0·39 inch.
1 millimetre (1 mm.)	= 0·039 inch = $\frac{1}{25}$ inch nearly.
1 μ (micron.)	= 0·001 millimetre = $\frac{1}{25000}$ inch nearly.

II.—SOLUBILITIES.

10 c.c. of a saturated alcoholic solution of methylene blue contain 0·068 grm. of methylene blue.

10 c.c. of a saturated aqueous solution of methylene blue contain 0·664 grm. of methylene blue.

10 c.c. of a saturated alcoholic solution of gentian violet contain 0·442 grm. of gentian violet.

10 c.c. of a saturated aqueous solution of gentian violet contain 0·175 grm. of gentian violet.

10 c.c. of a saturated alcoholic solution of fuchsin contain 0·292 grm. of fuchsin.

10 c.c. of a saturated aqueous solution of fuchsin contain 0·066 grm. of fuchsin.

10 c.c. of a saturated solution of corrosive sublimate contain 0·507 grm. of corrosive sublimate.

III.—BACTERIAL REMEDIES.

ANTITOXINS.

*General Principles of Antitoxin Treatment.*¹

A discussion on the utility of antitoxin treatment would be out of place in this manual. It is sufficient to say that in diphtheria the evidence in its favour is most striking; in septicaemia and puerperal fever antistreptococcic serum appears to have wrought some cures; and in tetanus, though many failures have been recorded, there have also been successes, and no other mode of treatment is so likely to directly modify the disease. As regards antitoxin in other diseases, enteric fever, cholera, tuberculosis, &c., it is still in the experimental stage.

One essential condition necessary for successful treatment with antitoxin is the use of the remedy at as early a period in the disease as possible. Antitoxin acts by rendering the cells and tissues insusceptible to the bacterial toxins, and if damage has already been done the effects of such damage will become apparent in spite of antitoxin. Antitoxin does not 'cure' the disease—in fact, strictly speaking there is no such thing as the healing or cure of a diseased condition by means of drugs or other treatment; it is the vital activities of the cells and tissues which do this. All that the physician or surgeon does by his treatment is to bring the damaged parts under such conditions, and to modify perverted cellular action in such a way, that the normal and regenerative vital activities of the living matter are placed under the most favourable conditions for bringing about repair. A damaged tissue is repaired by cellular action alone.

Antitoxic treatment is more likely to succeed in diphtheria than in some other diseases, for a local manifestation is usually present which enables the disease to be diagnosed at an early stage, before any quantity of toxin has been absorbed, whereas tetanus, for example, is only recognised by the occurrence of the results (spasms, &c.) of the tissue damage due to the tetanus

¹ This section is largely taken from a paper by the writer in *Treatment*, I. 1897, p. 173. See also the *Practitioner*, lv. 1895, p. 559.

toxins, and antitoxin treatment is therefore somewhat disappointing. Another essential condition is the administration of a sufficient amount of antitoxin, and this does not depend on the actual volume of serum injected. The antitoxic serum may be regarded as a solution containing a variable amount of antitoxin, and for therapeutic use its strength—that is, the amount of antitoxin present—must be ascertained. The strength of an antitoxic serum is for convenience described in arbitrary units, a unit being that amount of the serum which will completely neutralise ten lethal doses of a toxin injected into a 260-gramme guinea-pig.

The dose of antitoxin is dependent upon the gravity of the disease, and not on the age of the patient, for a slight consideration will show that just as much toxin may be formed in a child as in an adult. It has been found impracticable hitherto to separate the antitoxic constituent from the serum, so that attention has recently been directed to obtaining as strong an antitoxic serum as possible in order that the dose may be reduced in volume, and the diphtheria antitoxin can now be obtained containing the 1500 units in as little as 3 or 4 c.c.

In addition to antitoxin, general and local treatment should be pursued as usual in those diseases in which it is applicable, for antitoxin has no direct action on the bacteria which produce the disease. The antitoxins are strictly specific; diphtheria antitoxin, for example, has not the slightest influence in tetanus.

In cases of mixed infection, where the diphtheria bacilli are associated with streptococci or staphylococci, the diphtheria antitoxin may prove of less value, as it will have no influence on the streptococcic or staphylococcic infection. Likewise septicæmia and puerperal fever may be due to a variety of organisms, streptococci, staphylococci, *bacterium coli commune*, &c., but the anti-streptococcic serum is only of value when the infection is due to the streptococcus.

The complications and accidents of antitoxin treatment are few and usually unimportant. Abscess and other local troubles at the seat of inoculation should not occur if proper antiseptic precautions be taken. Urticaria or other rashes and joint pains are by far the most troublesome complications. At present these seem unavoidable and are to be treated on general principles, but

by employing stronger antitoxins, so that the amount of serum injected is less, they are not so marked. Accidents due to antitoxin treatment are happily very few, and are probably less frequent than with other drug treatment. For it must be remembered that there is a certain amount of risk with all drugs, and alarming complications and even fatal results are every year recorded from opium, potassium iodide, cocaine, and especially chloroform, but we do not on this account discard these valuable remedies.

Generally speaking the antitoxins keep unimpaired, certainly for several weeks, in a cool dark place. They diminish in strength by exposure to light and by warming. The bottle containing the antitoxin should only be opened at the moment of using. If any fluid is left it should be thrown away and *not* kept for subsequent administration.

There is perhaps some future for the use of antitoxins as prophylactics, as in outbreaks of diphtheria in schools or other institutions, and in some cases of wounds, in countries where tetanus is frequent. To sum up :—

1. Antitoxin to be of value must be used as early as possible—before tissue damage has occurred—and in a sufficient amount.

2. As antitoxin is *not* germicidal, it is well to employ local antiseptic treatment, where applicable, to the throat in diphtheria, to the wound in traumatic tetanus, and to the uterus in puerperal fever. General treatment also must not be omitted.

3. Antitoxins are specific and may fail in mixed infections, &c.

Diphtheria Antitoxin.—The amount of diphtheria antitoxin to be used depends upon its strength, which, as already explained, is reckoned in units.

Washbourn¹ recommends 2000 to 4000 units (according to the severity of the case) to be injected at once, and in severe cases the same dose may be repeated two or three times in the course of the ensuing twenty-four hours. If there is no improvement after three days' treatment further injections are not likely to do any good.

As soon as there is a reasonable probability that the case is one of diphtheria the antitoxin should be used, and the result of a bacteriological examination not waited for, as to be of service early treatment is of the utmost importance. If it does no good it will do no harm.

¹ *Treatment*, i. 23, 1898, p. 533.

Diphtheritic paralysis seems to be rather more frequent after the use of antitoxins, probably because a greater number of cases recover.

For prophylactic use 200 units should be injected. Immunity so produced does not last longer than about three weeks. (See also p. 177.)

Streptococcus Antitoxin.—The dose of streptococcus antitoxin is 10 to 20 c.c. every six, twelve, or twenty-four hours, according to the severity of the case. Some continental authorities consider this amount much too small and administer 50 to 150 c.c. for a dose. If no improvement follows after four or five injections as gauged by the temperature and general condition of the patient, further injections are not likely to do any good. It is only in cases of infection by the *streptococcus pyogenes* or *erysipielatis* that the serum will be of service.

N.B.—STREPTOCOCCUS ANTITOXIN RAPIDLY DIMINISHES IN STRENGTH WITH AGE AND SHOULD NOT BE KEPT.

Tetanus Antitoxin.—This remedy, although not so beneficial as diphtheria antitoxin, has apparently wrought a cure in many instances.

The dose is 10 to 20 c.c. every six or twelve hours, according to the gravity of the case. For prophylactic use, as in 'tetanus' districts, and in contused or lacerated wounds soiled with earth, 10 c.c. should be injected every fortnight for six weeks. In veterinary practice the expense of antitoxin treatment is prohibitive unless the animal be a valuable one. The dose is 20 to 40 c.c. In certain instances antitoxin may be used with advantage as a prophylactic in similar doses to those for man.¹

Pneumococcus Antitoxin.—The dose is 10 to 20 c.c. every twelve to twenty-four hours.²

Plague Antitoxin.—A dose of 10 to 20 c.c. repeated three or four times is stated by Yersin to have marked curative properties (see p. 267).

Other Antitoxins.—Several other antitoxins have been prepared, and the dose of all of them is 10 to 20 c.c. repeated every twelve or twenty-four hours. They are, however, still in the experimental stage, and some are probably of no value.

Such are typhoid (p. 234) and cholera (p. 249), yellow fever, tubercle and anthrax (p. 160), antitoxins, anticancerous sera (*a*, erysipelas antitoxin; *b*, a serum prepared by injecting animals with carcinomatous and sarcomatous tissues), antisyphilitic serum and *staphylococcus pyogenes aureus* and colon bacillus sera.

¹ See Hewlett, *Practitioner*, April, 1895, and Kanthack, *Med. Chron.* 1893.

² See Washbourn, *Brit. Med. Journ.* 1897, ii. p. 1849.

TUBERCULIN PREPARATION.

I. Koch's Original Tuberculin for Human Use.

The mode of preparation is described at p. 194. It is a thick amber-coloured fluid which has to be diluted for use. For diluting $\frac{1}{2}$ per cent. carbolic acid should be used, and only so much of the dilution prepared as can be used in a few days.

For the commencement of treatment a 1 per cent. solution can be used, later on a 10 per cent solution.

The following doses refer to the concentrated (i.e. undiluted) fluid:—

(a) The maximum initial dose for adults is 0·001 c.c. For children and in cases of visceral tuberculosis one-half to one-tenth of this amount should be given.

(b) In no case should the dose be repeated until the temperature has completely fallen to normal.

(c) If a severe reaction occurs, *i.e.* a rise of temperature of 3·5° F. and upwards, the dose should not be increased until the reaction produced by it does not exceed about 2° F.

(d) At the commencement of treatment, and when the dose is increased, at least a day should intervene between subsequent doses.

(e) The dose should be increased at first by 0·001 c.c. until 0·005 c.c. is reached; it can then be increased by 0·002 c.c., and from 0·01 c.c. a more rapid increase is permissible, the guide being the amount of reaction, temperature, &c. produced.

(f) In cases of lupus, the greatest amelioration is obtained by increasing the dose until 0·1 c.c. is reached, and the doses may be repeated three times a day.

(For doses, &c. see paper read by Watson Cheyne at the Royal Medical and Chirurgical Society, April 1891.)

(g) For diagnostic purposes the use of tuberculin is not to be recommended. In certain cases, however, it may be used, the dose being 0·005 c.c., and if this produces no reaction, 0·01 c.c. and 0·02 c.c.

The tuberculin is injected subcutaneously, the syringe and skin being disinfected with absolute alcohol.

Tuberculin for Veterinary Use.

In veterinary practice tuberculin is used almost exclusively for diagnostic purposes. The dose of the various preparations in the market varies according to their strength; it corresponds to 0·1 c.c. or 0·2 c.c. of Koch's original tuberculin.

(a) The dose is injected subcutaneously in the neck; the syringe and skin having been previously disinfected with 1—20 carbolic acid solution.

(b) If possible the temperature of the animal should be taken morning and evening for two or three days previous to inoculation.

(c) The temperature should be taken immediately previous to inoculation.

(d) The temperature should be taken at the twentieth hour after injection, or, where it can be done, at frequent intervals from the twelfth to the twentieth hour.

(e) The reaction consists of a rise of temperature of 1.5° to 6° F. above the average normal, occurring eight to twelve hours after injection, and lasting twelve to fourteen hours, accompanied by some systemic disturbance.

(f) A healthy animal is unaffected by the injection, and if an animal be very extensively affected with tuberculosis the reaction may not be given, or may be masked by the febrile condition present.

N.B.—IN CERTAIN INSTANCES TUBERCULIN MAY GIVE A REACTION IN OTHER DISEASES, *e.g.* SYPHILIS AND ACTINOMYCOSIS.

II. Koch's New Tuberculin.

1. Tuberculin-A, an alkaline solution of tubercle bacilli. Resembles the original tuberculin closely, but relapses are less frequent. An insuperable objection to its use is the formation of abscesses at the seat of inoculation when the doses become at all large, from the presence of dead bacilli in suspension.

2. Tuberculin-O closely resembles ordinary tuberculin. It does not produce abscesses, but is possessed of only very feeble immunising properties.

3. Tuberculin-R does not produce abscesses, possesses powerful immunising properties, and produces little or no reaction in tubercular subjects unless the dose be large. This seems to be the most suitable preparation to employ in treatment, and it is to be administered by subcutaneous injection in the same manner as the original tuberculin. The liquid contains ten milligrams of solid matter per cubic centimetre. For use it must be diluted with normal salt solution (0.7 per cent.) and not with carbolic acid solution. The first dose should contain not more than $\frac{1}{1000}$ of a milligram of solid matter, *i.e.* 0.2 c.c. of a dilution of 1 : 1000. This amount should produce no marked reaction; if it does initial doses must be still further reduced. The injections are repeated about every other day. The dose is slowly increased so as to avoid as far as possible a rise of temperature above 1° C. Should an injection

be followed by a rise of temperature this must be allowed to fall to normal before giving the next injection. The treatment is pursued until a dose of twenty milligrams of solid matter is reached (*i.e.* 2 c.c. of the undiluted preparation). The treatment must not be begun too late; it is useless when the disease is advanced and a fatal termination imminent. In mixed infections also little benefit is to be expected. This is generally the case in patients whose temperature rises above 38° C. In lupus the cutaneous lesion improves, although local reaction is slight. In phthisis the early injections are followed by a slight increase in the crepitant râles. After a few injections the râles disappear and cough and expectoration diminish and ultimately cease. No alarming symptoms or untoward results were met with, and the temperature of patients who show a daily rise becomes normal throughout. In a number of favourable cases considerable improvement occurred in all, an improvement so marked that for the time being, at any rate, it might be termed a cure (Koch).

MALLEIN.

The mode of preparation of mallein has been described at p. 219. It is used for the diagnosis of glanders in the horse, and is most valuable. It has but slight, if any, curative properties.

(a) The dose is to be injected subcutaneously in the neck over the vertebræ, about midway between the jaw and shoulder; the syringe and skin having been previously disinfected with 1—20 carbolic acid solution.

(b) If possible the temperature of the animal should be taken morning and evening for two or three days previous to inoculation.

(c) The temperature should be taken at the twentieth hour after injection, or, where it can be done, at frequent intervals from the twelfth to the twentieth hour.

(d) A complete reaction comprises:—

1. A rise of temperature of more than 2·7° F.
2. An extensive hot and painful swelling at the seat of inoculation.

(e) Systemic disturbance, such as prostration, loss of appetite, shivering, &c. may occur. These symptoms, when present, are more or less persistent.

(f) The temperature reaction is unreliable in all cases where the temperature at time of inoculation is 2·5° F. above normal. In such cases, if there are any suspicious clinical signs to assist, reliance may be placed on the occurrence of the local swelling.

VACCINES.

These are for prophylactic use only; and are of no value for curative purposes.

1. VACCINE LYMPH.—There is no need to say anything about vaccination, except that probably in the future glycerinated calf lymph will be exclusively employed.

2. ANTI-CHOLERA VACCINE.—The vaccines employed in the anti-choleraic vaccination are two in number, a first or weak, and a second or strong. The following is Haffkine's method¹:—

The first vaccine is prepared from attenuated cultures of the cholera spirillum. The ordinary laboratory cultures are usually considerably attenuated, but to be sure that they are sufficiently so they are grown for several generations on surface agar at 38° C. in tubes through which a current of moist air is continuously passed. Such a culture causes only a local cedema instead of necrosis when injected into the subcutaneous tissue of a guinea-pig.

The second, or strong vaccine, is prepared from cholera cultures, the virulence of which has been artificially increased by growing in the peritoneal cavity of guinea-pigs. This is done by first of all preparing standard cultures from any ordinary culture of cholera bacilli. Test-tubes measuring 15 cm. in length are employed; of the 15 cm., 10 cm. are occupied by the sloping surface of ordinary nutrient agar. The whole surface of the nutrient medium is inoculated and the inoculated tubes are incubated at 35° C. for twenty-four hours. The whole growth from the surface of the agar is then scraped off with a sterilised platinum needle of stout wire and made into an emulsion with about 3 c.c. of sterile broth. A guinea-pig (300–400 grm.) is etherised, a small patch of hair on the abdomen cut short, and a spot cauterised with a hot iron to sterilise it. The emulsion of cholera bacilli is then drawn up into a sterile syringe or glass pipette and injected into the abdominal cavity through the cauterised area. Two guinea-pigs should be injected at the same time, using for each one a standard cholera culture. The guinea-pigs so treated will die within twenty-four hours.

The animals are then pinned out and the peritoneal cavity opened with strict aseptic precautions; with a sterile forceps the intestines are thrown upwards and to the right, and with a sterile glass pipette the peritoneal fluid is sucked up from the iliac fossæ. The whole of the peritoneal fluid from one guinea-pig is introduced into a sterile test-tube which is well plugged with cotton-wool and placed in the

¹ *Brit. Med. Journ.* 1893, i. p. 227 (Wright and Bruce).

oblique position (for aëration) in the incubator at 35° C. for about ten hours. This is to allow of the proliferation of the cholera bacilli. After this treatment the fluid is similarly injected into a second guinea-pig, the size of which, however, has to be taken into account. If the peritoneal fluid in the first guinea-pig be abundant it will contain comparatively few cholera bacilli, and a smaller animal should be chosen, but if it be scanty the comma bacilli will be numerous, and a larger animal may be used. After twenty to thirty passages through guinea-pigs the virus will have attained its maximum virulence, which is known by the fact that further passages do not shorten the period which elapses between inoculation and death.

Throughout all the manipulations the greatest care must be taken to prevent contamination, and the cultures, &c. should be controlled by subculturing and microscopical examination. The 'exalted' cholera cultures do not retain their maximum virulence for longer than ten days, and have again to be passed through guinea-pigs (three or four).

In order to prepare the vaccines a 'standard' agar tube is inoculated over its whole surface and incubated at 35° C. for twenty-four hours. Three or four cubic centimetres of sterile broth are introduced into the tube and an emulsion is made with the whole of the growth. The emulsion is measured by drawing it up into a sterile syringe, the contents of which are then introduced into another sterile glass and made up to a volume of 8 c.c. by the addition of more sterile broth. One cubic centimetre of this emulsion constitutes the dose for vaccination. Carbolised vaccines may be prepared by using a $\frac{1}{2}$ per cent. solution of carbolic acid (sterilised by boiling) for making the emulsions and diluting them to 6 c.c., and not 8 c.c., as in the uncarbolised. The carbolised vaccines may be preserved some time in sealed tubes.

The dose of vaccine (1 c.c.) is injected hypodermically into the flank, the second or strong vaccine being injected three to five days after the first or weak one.

3. ANTI-TYPHOID VACCINE.—The following is an abstract of a paper by Wright & Semple¹ :—

Method of preparing the Anti-Typhoid Vaccines.—Agar cultures of typhoid bacilli grown for twenty-four hours at blood-heat are employed and the cultures thus obtained are emulsified by adding measured quantities of sterile broth to them. This emulsion is drawn up into a number of sterile and calibrated glass pipettes, the capillary ends of which are sealed up in the flame, so forming vaccine capsules. These capsules are placed in a beaker of cold water, which is then heated to 60° C., and they remain in it for five minutes. The sterility of the

¹ *Brit. Med. Journ.* 1897, i. p. 256 (Full Refs.).

vaccines is then tested by allowing a drop to run out on to the surface of an agar tube, which is afterwards incubated, and if the contents of the capsules prove sterile the vaccine is ready for use.

The Dosage and Strength of the Anti-Typhoid Vaccines.—The strength of a typhoid vaccine depends upon the number of bacilli it contains and their virulence. The culture employed was of such a strength that one-fourth tube of a twenty-four hour culture was a lethal dose for a 350 to 400 grm. guinea-pig when inoculated hypodermically. The dose of the vaccine varies from one-twentieth to one-fourth of a tube. The latter is a somewhat severe dose for a man and would prove fatal to a 350 grm. guinea-pig.

Clinical Symptoms which supervene upon the Inoculation of the Anti-Typhoid Vaccines.—The symptoms are comparatively slight when small doses are used (one-twentieth to one-sixth of a tube), such as tenderness at the seat of inoculation, and two or three hours after inoculation a chilly feeling, slight rise of temperature, and restlessness at night, but these symptoms pass away in about twenty-four hours. With the larger doses all the symptoms are severe, and are described as commencing two or three hours after injection with tenderness, which gradually increases in severity and extends upwards into the armpits and downwards into the groin, a patch of congestion two or three inches in diameter develops round the site of inoculation, and red lines of inflamed lymphatics can be traced extending into the armpits. These symptoms gradually subside in about forty-eight hours.

The constitutional symptoms were marked by some degree of faintness and collapse, in some cases accompanied by nausea and vomiting, which commenced about three to four hours after injection, entire loss of appetite, disturbed sleep, and high temperature, all of which passed off in a few hours. The blood and serum of individuals vaccinated in this way give the Widal reaction in a marked manner.

Probably to obtain a complete protection three or four successive vaccinations, with increasing doses, at intervals of one to two weeks, would be advisable.

There is practically no risk, and the immunity conferred would probably last for some years.

As regards the sphere of application of the anti-typhoid vaccination, it might be expedient in the case of young soldiers or other individuals going abroad to infected districts, for those living in a district visited by an epidemic, or for those in attendance on a typhoid patient.

4. ANTI-PLAGUE VACCINE. See p. 267.

COLEY'S FLUID.

This preparation consists of the toxins of the streptococcus of erysipelas and the *bacillus prodigiosus*. It was devised by W. B. Coley, of New York, as a possible cure for inoperable malignant tumours. The treatment is based on the undoubted fact that malignant growths may decrease or even disappear completely after an attack of erysipelas. The fluid is prepared by growing the streptococcus, obtained from a fatal case of erysipelas and rendered highly virulent by a succession of passages through rabbits, in bouillon for about ten days; the *prodigiosus* is now added and the two are allowed to grow together for another week or ten days. The culture is then heated to from 58° to 60° C. for one hour and a piece of thymol added to keep it.

The fluid is injected subcutaneously in the vicinity of the tumour. The earlier injections may be performed with the *filtered* toxin, which does not produce so much reaction as the unfiltered.

The dose to commence with should be 1 to 2 minims of the filtered, or $\frac{1}{2}$ minim of the unfiltered, fluid. The dose is gradually increased each day until there is a temperature reaction of 103° to 104° F. The temperature is the chief guide in estimating the dose, and the frequency of injections depends upon the general condition of the patient and upon the rapidity of recovery from the depression of the preceding dose. The injection must not be repeated until the temperature has completely fallen.

Coley has treated ninety-four cases of sarcoma and sixty-three cases of carcinoma with his fluid. Of the sarcomata about half (forty-five) showed more or less improvement, the greatest benefit being obtained in the spindle-celled variety, less in the round-celled, hardly any in osteo-sarcoma, and none in the melanotic. At least twelve cases appear to have been permanently cured.

The treatment does not appear to be so successful in carcinoma, only two or three cases having been permanently cured.

Coley himself advocates the treatment only in inoperable cases.

Literature.

- Coley, *Amer. Journ. of Med. Sc.* 112, 1896, p. 251.
Coley, *Johns Hopkins Hosp. Bull.* vii. 1896, p. 157.
Coley, *Ann. of Surgery*, xxv. 1897, p. 174.
Coley, *Ann. of Surgery*, xxvi. 1897, p. 232.

NUCLEINS.

The nucleins derived from yeast cells, from the testis, and thymus are powerful germicides, and have been advocated for the treatment of

tuberculosis. Yeast nuclein, perhaps the best preparation, is obtained by thoroughly washing yeast by decantation and sedimentation, then digesting with pepsin and dilute hydrochloric acid. The undissolved residue is the nuclein, which is further purified by repeated solution in dilute alkali and precipitation by dilute acid. (See also p. 120.)

DE BACKER'S METHOD.

Said to be of service in tuberculosis and cancer. It consists of a pure culture of yeast, which is stored under pressure in glass vessels, like syphons, provided with a hollow needle. The exact mode of preparation does not seem to be public.¹

IV.—MAKERS OF APPARATUS, &c.

Microscopes.

Messrs. Baker & Co., High Holborn, W.C.
 Messrs. Powell & Lealand, Euston Road, N.W. (lenses).
 Messrs. Swift & Son, Tottenham Court Road, W.C.
 Messrs. Watson & Son, High Holborn, W.C.
 Messrs. Leitz; Agency—Messrs. Baker & Co.
 Messrs. Winkel, Göttingen, Germany.
 Messrs. Carl Zeiss; Agency—Margaret Street, W.

Incubators.

Messrs. Baird & Tatlock, Cross Street, Hatton Garden, E.C.
 Messrs. Hearson & Co., Regent Street, W.

Stains, Cover-glasses, Slides, &c.

Messrs. Baird & Tatlock.
 Mr. Kanthack, Berners Street, W. } Agents for Grubler's Stains.
 Messrs. Baker & Co.

Reagents, Chemicals, Apparatus, &c.

Messrs. Baird & Tatlock.
 Mr. Kanthack.
 Messrs. Townson & Mercer, Bishopsgate Street, E.C.

¹ See *Brit. Med. Journ.* 1897, ii. p. 802.



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